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Abstracts

1. Cyclic AMP potentiation of insulin release from pancreatic β cells is not mediated by an increased cytosolic Ca^{2+} activity

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The effect of cyclic AMP on the cytosolic Ca^{2+} activity (Ca^{2+}_i) in pancreatic β-cells isolated from *ob/ob*-mice was investigated using the quin-2 technique. In the unstimulated β-cell Ca^{2+}_i was 160 nmol/l. When insulin secretion was stimulated with 20 mmol/l D-glucose there was an increase of Ca^{2+}_i by approximately 40%. A subsequent addition of 5 μmol/l forskolin to elevate intracellular cyclic AMP levels did not influence Ca^{2+}_i despite a doubling of insulin secretion. Qualitatively similar results were obtained with dibutyryl cyclic AMP (2 mmol/l) and theophyllin (1 mmol/l). The β-cells remained responsive throughout the experiment since depolarization of the β-cells with excessive K^+ at the end of the experiment induced a rapid and sizeable increase of Ca^{2+}_i. Quin-2 neither affected the glucose metabolism nor the glucose-stimulated insulin release of the β-cells. The results indicate that cyclic AMP potentiates insulin release by sensitizing the secretory mechanism of the β-cells to Ca^{2+} without greatly affecting Ca^{2+}_i.

2. The biological activity of subcutaneously injected biosynthetic human proinsulin and human insulin zinc suspension

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The biological activities of biosynthetic human proinsulin and human insulin zinc suspension (IZS) were compared by a 24 h glucose clamp method with those of human soluble and isophane (NPH) insulins. Seven normal volunteers received soluble insulin and proinsulin; five also received NPH and IZS. Injections were given into the anterior abdominal wall in random order, one week apart, at a dose of 0.5 U/kg body weight (proinsulin standardized at 4 U/mg). After soluble insulin, proinsulin, NPH and IZS, peak glucose requirements were 12.19 ± 0.74, 9.93 ± 0.69, 9.0 ± 1.45 and 5.08 ± 1.18 mg·kg^{-1}·min^{-1} (mean ± SEM) at 4.29 ± 0.64, 5.57 ± 0.57, 6.4 ± 0.93 and 11.0 ± 1.82 h respectively. Requirements returned to zero at 10.71 ± 0.75, 14.43 ± 0.78, 18.4 ± 1.63 and 20.4 ± 1.6 h. Total glucose infused over 24 h was 4.32 ± 0.26 g·kg^{-1}·day^{-1} for soluble insulin, compared with 4.67 ± 0.44, 4.38 ± 0.77 and 2.93 ± 0.54 g·kg^{-1}·day^{-1} for proinsulin, NPH and IZS. IZS had a slower onset than the other preparations including NPH ($p < 0.01$) and a longer duration of action than proinsulin ($p < 0.05$). Proinsulin had a slower onset ($p < 0.025$) and longer duration of action ($p < 0.01$) than soluble insulin; its profile falling between those of soluble and NPH. This time course may prove clinically useful, particularly for twice daily injection regimes.

3. Importance of the route of intravenous glucose delivery to hepatic glucose balance

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To assess the importance of the arterial-portal glucose gradient (A-PGG) in determining hepatic glucose balance (HGB) eight conscious overnight-fasted dogs were given glucose intravenously via a portal or peripheral route. Insulin and glucagon were held constant and basal [somatostatin + intraportal insulin (184 μU·kg^{-1}·min^{-1}) and glucagon (0.65 ng·kg^{-1}·min^{-1})]. HGB was measured using the arterio-venous difference technique during a control and two 90 min glucose infusion periods. The sequence of infusions was randomized such that half of the dogs received glucose via the portal route first. Insulin, glucagon and hepatic blood flow were similar during the control, portal and peripheral infusion periods (7 ± 1, 7 ± 1, 7 ± 1 mU/l, 100 ± 3, 101 ± 6, 101 ± 3 ng/l and 37 ± 1, 34 ± 1, 32 ± 3 ml·kg^{-1}·min^{-1}, respectively). In the three periods the glucose infusion rate, arterial glucose level, hepatic glucose load, A-PGG and HGB were 0 ± 0, 25 ± 3, 24 ± 2 μmol·kg^{-1}·min^{-1}; 4.4 ± 0.2, 8.6 ± 1.0, 9.2 ± 0.9 mmol/l; 157 ± 7, 315 ± 8, 308 ± 18 μmol·kg^{-1}·min^{-1}; $+0.09 \pm 0.04$, -0.94 ± 0.12, $+0.18 \pm 0.04$ mmol/l and $+12.1 \pm 1.7$, -8.8 ± 2.2, $+0.6 \pm 1.2$ μmol·kg^{-1}·min^{-1}, respectively. Thus, when glucose was delivered via a peripheral vein the liver did not take up glucose, but when a similar load was delivered intraportally the liver took up 32% of it ($p < 0.01$). In conclusion, the arterial-portal glucose gradient may be a crucial signal for hepatic-peripheral distribution of a glucose load.

4. Cysteamine and the endocrine pancreas in the mouse

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Cysteamine has been shown to reduce the somatostatin content in pancreatic somatostatin cells. Cysteamine may thus be of potential value in studying the possible paracrine influences of somatostatin on islet function. A combined immunohistochemical and functional study was therefore performed on the effects of cysteamine on the endocrine pancreas in the mouse. Cysteamine (300 mg/kg subcutaneously), transiently reduced somatostatin 14-like immunostaining in somatostatin cells. Virtually no immunostaining was seen 4 h after injection. After 28 h, a partial and after 52 h a complete return was observed. In contrast, somatostatin-28-like immunostaining was not affected by cysteamine, and also insulin and glucagon immunostaining were unaffected. Cysteamine transiently decreased plasma glucose concentration. By 1 h after injection, it was 7.1 ± 0.4 mmol/l compared with 10.1 ± 0.3 mmol/l in control mice. The reduced plasma glucose levels gradually returned to normal within 28 h. Plasma insulin levels were unaffected apart from a slight depression seen 1 h after injection of cysteamine. In alloxan-diabetic mice cysteamine also reducedplasma glucose levels. In conclusion, cysteamine affects islet somatostatin cells, perhaps influencing the formation of somatostatin-14 from the somatostatin precursor. Also, plasma glucose concentrations are reduced by an insulin independent action.

5. The insulin dosage computer: a new approach to optimizing conventional insulin therapy

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Control in diabetes is undeniably important. But frequent medical intervention may not always be practical and for some patients intensified treatment methods may not always be acceptable. For such patients who nonetheless are prepared to estimate capillary blood glucose levels up to four times a day while following a regular lifestyle and diet, we have developed a tiny computer. It weights 200 g and can receive, store and analyze these data. Twice daily it recommends changes in short- and intermediate-acting insulin according to a strategy aimed at reducing the four pre-meal capillary blood glucose levels toward normal levels of 6.1 mmol/l. Seven Type I (insulin-dependent) diabetic outpatients ranging in age from 11 to 49 years used these devices for 60 days. Before starting their mean ± SD capillary blood glucose levels were respectively 9.9 ± 2.9, 10.4 ± 5.1, 11.2 ± 3.2, 11.5 ± 1.9 mmol/l. After 60 days, the corresponding values had all fallen significantly: 6.4 ± 1.4, 6.1 ± 0.9, 8.2 ± 2.3, 7.5 ± 1.3 mmol/l ($p < 0.05$). There was also a significant reduction in the variability ($p < 0.01$). HbA_1 fractions fell in each patient by 1–3% ($p < 0.005$). Changes in lifestyle were not necessary or encouraged. Individual insulin dosages were strategically modified but only the mean ± SD intermediate insulin at breakfast was increased significantly from 28 ± 8 to 39 ± 12 U ($p < 0.05$). We conclude that a microprocessor-based device can elegantly exploit four daily capillary blood glucose estimates and as often as twice-daily recommend changes in insulin therapy, that these dosage changes improve glycaemic control (by reducing both mean glycaemia and its variability, as well as the HbA_1 fraction), and finally, that the reality of an *insulin dosage computer* provides both the diabetic patient and his physician with a new approach to optimizing conventional insulin therapy.

6. Effects of immunoglobulin and sera from newly diagnosed Type 1 (insulin-dependent) diabetic patients on glucose-induced insulin release in human islets of Langerhans

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Immunoglobulin (IgG) deposition in islet cells has been established in the pancreases of newly-diagnosed diabetic children, implying internalization of membrane bound islet cell antibodies (ICA). To determine the possible biochemical role of membrane-bound ICA on human islet cell function, sera from 50 children with newly-diagnosed Type 1 diabetes were screened for various types of ICA using frozen sections of human pancreas. Heat-inactivated ICA-positive serum samples were pooled according to the type of ICA present, and half of these pools fractionated on DEAE Sephadex to obtain purified IgG and IgM immunoglobulin fractions. Both sera and purified immunoglobulin were examined for their effects on insulin biosynthesis and secretion in islets, using both perifusion and static incubation in the presence of varying concentrations of glucose (2, 8, 12, 20 mmol/l). Results indicate an increase in the basal rates of insulin release at glucose (2 and 8 mmol/l) with ICA sera and a decrease in insulin release with purified immunoglobulin. Both sera and immunoglobulin indicate a significant decrease in stimulated insulin release at glucose (12 and 20 mmol/l), with the latter giving an almost complete inhibition.

7. Absorption of oral glucose load in man is delayed by peptide YY

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Peptide YY (PYY) has recently been identified within mucosal, endocrine cells of the lower gastro-intestinal tract. The effect of low dose infusion of this peptide, mimicking the level seen after a large meal, on the insulin response to a glucose load and the rate of gastric emptying was determined in five healthy male volunteers. Each subject received, in random order, saline and natural porcine PYY (2 pmol · $kg^{-1} \cdot min^{-1}$) infusion. After 20 min, the subject drank 50 g glucose in 200 ml water. The rise in plasma glucose was significantly impaired during the infusion of PYY (30 min: plasma glucose 5.44 ± 0.52 pmol/l) compared with saline (7.14 ± 0.72 pmol/l). In association with this, the response of plasma insulin was also impaired during the infusion of PYY (incremental integrated response of insulin during PYY infusion 4737 ± 965 pmol/l per 75 min compared with saline infusion 8741 ± 1056 pmol/l per 75 min). Gastric emptying measured by the rate of disappearance of ^{99m}Tc-tin colloid from the stomach was significantly delayed by the infusion of PYY.

As motility of the upper gut is of major importance in the control of the rate of absorption of nutrients, the effect of PYY in delaying gastric emptying and thus reducing the rise in plasma glucose after an oral glucose load may have an important role in carbohydrate metabolism.

8. Glucose, insulin, and C-peptide levels during a 75 g oral glucose tolerance test in healthy man

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Although the amount of glucose recommended for performing an oral glucose tolerance test (GTT) is 75 g, there are few studies that report data on the 75 g oral GTT. Thus, we have measured plasma glucose, insulin, and C-peptide levels during a 2-h 75 g oral GTT in 247 healthy ambulatory volunteers (134 males, 113 females) aged 13–69 years. Subjects under study had normal body weight (> 80 < 120% ideal body weight), normal glucose tolerance, normal blood tests, normal physical examination, no family history of diabetes, no history of important diseases, and were not consuming drugs. Results (mean ± SEM, range): fasting glucose 4.64 ± 0.03 mmol/l (3.10–6.10), 1-h glucose 5.23 ± 0.10 mmol/l (2.20–9.90), 2-h glucose 4.11 ± 0.06 mmol/l (2.00–6.80); fasting insulin 0.088 ± 0.002 nmol/l (0.030–0.28), 1-h insulin 0.45 ± 0.01 nmol/l (0.060–1.63), 2-h insulin 0.24 ± 0.01 nmol/l (0.050–1.12); fasting C-peptide 0.60 ± 0.15 nmol/l (0.14–1.34); 1-h C-peptide 2.17 ± 0.05 nmol/l (0.63–8.56), 2-h C-peptide 1.77 ± 0.04 nmol/l (0.35–5.74). A comparison made between 80 males and 80 females who were comparable for age and identical for body weight showed fasting glucose, 1-h glucose and C-peptide levels significantly higher in males than in females. The study of partial coefficients of correlation between age of subjects and parameters under study showed positive relationships between age and 1-h glucose, insulin, and C-peptide levels as well as 2-h C-peptide concentrations.

9. Absence of insulin receptor down-regulation in rat hepatocytes during fetal life in rats

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Although insulin regulates its own receptor concentrations during adult life, the elevated levels of circulating insulin and of insulin receptor concentrations in the fetus suggest that such an auto-regulatory mechanism is not present in the embryo. To evaluate this hypothesis, isolated hepatocytes from 21 day-old-rat-fetuses (21-F) and adult rats were pre-incubated with or without insulin (10^{-9} to 10^{-6} mol/l) for varying periods of time (0–8 h), thoroughly washed to remove reversibly bound insulin, and then incubated with ^{125}I-insulin to determine receptor concentration. In adult rats insulin induced a significant loss of receptor number which was proportional to the hormone concentration used. In 21-F, insulin was without effect at any concentration or time of pre-incubation. After 6 h pre-incubation with or without 10^{-6} mol/l insulin, hormone binding (fmol/10^6cells) in adult rats was 4.60 ± 0.26 and 2.22 ± 0.15, respectively, while, in 21-F it was 1.84 ± 0.45 and 1.88 ± 0.34. Studies of the kinetic properties of the insulin receptor of adult rats and 21-F revealed that association and dissociation rates were indistinguishable. Hormone degradation did not account for differences observed in binding. These results indicate the absence of insulin receptor down-regulation in fetal hepatocytes which could thus favour the anabolic processes of the embryo.

10. The rate of glucose fall does not determine the counter-regulatory hormone response to hypoglycaemia

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To examine whether the rate of glucose fall affects the counter-regulatory hormone response to hypoglycaemia, 12 normal subjects received intravenous insulin (1 mU/$kg^{-1} \cdot min^{-1}$) plus a variable glucose infusion. In six, plasma glucose fell rapidly from 4.6 to 2.9 mmol/l over 10 min; in six, glucose was gradually reduced (from 4.6) by 0.017 mmol · $l^{-1} \cdot min^{-1}$, reaching 2.9 mmol/l at 100 min. The same hypoglycaemic level (2.9 mmol/l) was maintained for 1 h in both groups. Increments in counter-regulatory hormones were no greater in the fast fall studies than in the slow fall group: adrenaline (1837 ± 358 versus 1678 ± 374 pmol/l); noradrenaline (401 ± 124 versus 395 ± 41 pmol/l); cortisol (167 ± 56 versus 278 ± 56 nmol/l) and growth hormone (19.9 ± 9 versus 22 ± 2 µg/l). Indeed, glucagon rose less with the fast fall (65 ± 14 versus 157 ± 38 ng/l, $p < 0.05$). The glucose level which triggered hormone responses was 3.3 ± 0.1 mmol/l. In conclusion, (1) counter-regulatory hormone responses to hypoglycaemia are not exaggerated by increasing the rate of glucose fall but

are determined by glucose level; (2) glucose thresholds for counter-regulatory hormone release are within the normal post-prandial range and (3) discrepancies between blood glucose levels and hypoglycaemic symptoms should probably not be attributed to the rate of glucose fall.

11. In vitro potentiation of glucose-induced insulin release by thiols: significance of cysteine compounds

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It has been suggested that the insulin-releasing capacity of glucose depends on islet thiols. Collagenase-isolated islets were incubated in Krebs-Ringer buffer containing glucose (11.1 mmol/l) and sulphydryl compounds. Insulin released into the medium was measured after 10 min of incubation by radioimmunoassay. L-cysteine, N-acetyl-D-penicillamine, L-cysteine-methylester, L-cysteine-ethylester, D-penicillamine, N-acetyl-L-cysteine, cysteamine and glutathione augmented glucose-induced insulin secretion in a dose-related manner. Their maximal effect occurred in the presence of 6, 2, 1, 1, 0.5, 0.1, 0.1 and 0.1 mmol/l, respectively. Na-2-mercapto-ethansulphonate, 2-mercaptopropionylglycine, meso-2.3-dimercapto-succinic acid, 2.3-dimercapto-l-propanol and the disulphides L-cystine, cystamine and D-penicillamine-disulphide as well as the thioethers, S-carboxymethyl-L-cysteine and S-carbamoyl-L-cysteine, were without effect. Our data suggest that only those sulphydryl compounds which contain cysteine augment glucose-induced insulin secretion. As far as the cysteine molecule is concerned, its effect occurs only if the free thiol group is available. On the other hand, the presence of a carboxylic group in the cysteine molecule appears not to be essential.

12. Elevated vasoactive intestinal polypeptide content of the sciatic nerve in diabetic rats and increased VIP accumulation after nerve transection

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We have previously demonstrated a marked accumulation of vasoactive intestinal polypeptide (VIP) in the proximal stump of the sciatic nerve after transection in rats: in contrast, there is a depletion of substance P. We now report that 10 weeks after streptozotocin-induced diabetes in adult Wistar rats, there was a significant elevation of VIP content of the intact sciatic nerve (control rats 1.8 ± 0.7 pmol/g, $n = 5$; diabetic rats 4.2 ± 0.8, $n = 6$; $p < 0.05$, Student's t-test). Furthermore, 2 weeks after sciatic transection, there was increased accumulation of VIP in the nerve stump of the chronically diabetic rats (controls 10.7 ± 0.9, diabetics 17.3 ± 4.4 pmol/g); this also occurred in segments of the sectioned nerves taken proximal to the neuromas (controls 10.2 ± 0.9, diabetics 13.5 ± 0.6 pmol/g, $p < 0.05$). Diabetes did not affect substance P and somatostatin levels in intact nerves, or their decreases after injury. As increased numbers of VIP-staining fibres were visible in the neural connective tissues after neurectomy, and VIP is a potent oedema potentiator, the VIP increases may relate to the known over-hydration and endoneurial oedema in the peripheral nerves of diabetic rats. Defects of axonal transport in diabetes appear here not to retard VIP accumulation.

13. Monitoring kindney function in insulin-dependent diabetic patients with nephropathy

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Glomerular filtration rate (GFR, single bolus ^{51}Cr-EDTA technique), serum creatinine and serum β_2-microglobulin concentrations were measured simultaneously in 49 insulin-dependent diabetic patients with nephropathy. GFR ranged from 148 to 23 ml/min per 1.73 m^2. Inverse serum concentrations of creatinine and β_2-microglobulin showed a significant correlation with GFR over the whole range of values ($r = 0.87$ and $r = 0.90$, respectively; $p < 0.001$). However, in the 31 patients with a GFR < 80 ml/min per 1.73 m^2, serum concentrations of creatinine and β_2-microglobulin were within the normal range in 12 and 9 patients, respectively. A prospective study for up to 70 months was performed in 18 of the patients. The study revealed a closer relationship between the individual rate of decline in GFR and of inverse serum β_2-microglobulin compared to GFR versus inverse serum creatinine levels ($p < 0.01$). Our cross-sectional study does not support the suggestion that serum β_2-microglobulin is a sensitive and reliable method in screening for early impairment of the kidney function in diabetic nephropathy. However, our prospective study suggests that the inverse of β_2-microglobulin concentrations is a simple and reliable method for monitoring glomerular function in individual patients.

14. Cyclosporin A inhibits cell replication in cultured mouse pancreatic islets

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In the light of recent attempts to treat newly-diagnosed Type 1 (insulin-dependent) diabetic patients with cyclosporin A (CsA) and reports suggesting an impaired glucose tolerance following immunosuppression therapy with CsA, we investigated the long-term effects of CsA on islet B-cell morphology and function in vitro. Collagenase-isolated mouse pancreatic islets were cultured free-floating for 7 days in medium RPMI 1640 + 10% calf serum in the presence of CsA (0.1 or 1.0 μg/ml). Islets cultured in the presence of the higher CsA concentration had an impaired islet proinsulin biosynthesis and insulin release when challenged with high glucose. Moreover the insulin and total RNA content of the drug-exposed islets was decreased as was the rate of DNA synthesis. The glucose oxidation and respiratory rates remained unaffected, suggesting that impaired insulin production was not a result of defective oxidative islet metabolism. There were no changes in the ultrastructure or phospholipid biosynthesis of the islets after the drug treatment. These data indicate that CsA affects islets in culture, although the clinical implications of this cannot be stated at present. The inhibitory effect of CsA on islet cell DNA synthesis must nevertheless be considered in attempts to ameliorate Type 1 diabetes and when grafting islet cells in numbers insufficient to provide a primary cure for the recipient.

15. Effects of glucose on insulin gene expression

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Prolonged growth of a transplantable insulinoma in inbred NEDH rats produces atrophy and functional suppression of the host pancreatic β cells. After tumour ablation, a transient 2–3 day interval of insulinopenia and hyperglycaemia is observed and restoration of β cell number, morphology and function is achieved at 5 days. To evaluate the role of glucose in modulating these changes, 50% dextrose was infused intravenously to tumour-bearing rats ($n = 15$) for 1–3 days using an indwelling cardiac catheter. Electron microscopic, autoradiographic and morphometric analyses characterized β-cell replication and islet cell changes during the infusion period. In addition, proinsulin mRNA concentrations were determined from autoradiographs obtained by filter hybridization to a cloned rat proinsulin I cDNA probe. Maintenance of glycaemia in excess of 8.3 mmol/l, despite profound hyperinsulinaemia (200 mU/l), resulted in hypertrophy, enhanced insulin biosynthesis and ^{3}H-thymidine incorporation of the pancreatic β cells by 3 days. Proinsulin mRNA levels markedly increased after 2 days of infusion and preceded β cell replication. These data suggest that glucose stimulates transcription of the insulin gene and replication of the β cell. In addition, the results suggest that β-cell suppression results from tumour-induced hypoglycaemia.

16. Suppression of the dawn phenomenon by somatostatin

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Five Type 1 (insulin-dependent) diabetic patients were connected on two different days to an artificial pancreas in an attempt to elucidate the pathogenesis of the 'dawn phenomenon'. From 21.00 h onwards, after 12 h of blood glucose normalisation, they received somatostatin 250 μg/h and glucagon 2 ng · kg^{-1} · min^{-1}, or saline infusion, for 12 h. Continuous blood sampling allowed the determination of hourly integrated free insulin, glucagon, growth hormone (GH) and cortisol levels. Somatostatin + glucagon infusion resulted in a complete suppression of GH levels and in a twofold increase of peripheral glucagon concentrations as compared with saline. Cortisol rhythm was not affected by somatostatin. Significant ($p < 0.02$) increases of the insulin infusion rates given by the artificial pancreas (0–4 h: 15 ± 4; 4–8 h: 25 ± 4 mU · kg^{-1} · h^{-1}) and of the insulin clearance rates (0–4 h: 273 ± 66; 4–8 h: 387 ± 92 ml/min per m^2) were observed during saline infusion. These increases were totally blunted by somatostatin + glucagon administration. Our results show that cortisol rhythmicity is not involved in the pathogenesis of the 'dawn phenomenon'. The nocturnal GH peak, with its delayed insulin-antagonistic effect, might account for this increase of insulin requirements during the early morning hours in Type 1 diabetic subjects.

17. Regional variations in insulin action in human adipose tissue during fasting

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Reginal variations in fasting-mediated changes in insulin action were looked for in femoral, gluteal and abdominal adipose tissue of 23 obese but otherwise healthy subjects. They were investigated before and after total fasting for 7 days. Fasting significantly increased high affinity insulin receptor binding in gluteal fat cells. However, fasting did not alter insulin binding in femoral or abdominal adipocytes. Insulin stimulated glucose oxidation in all three adipose regions before fasting in a dose-dependent way. Half maximum insulin effect was 150–250 U/l. In femoral and gluteal sites after fasting the amplitude of the insulin effect on glucose oxidation was significantly decreased, but there was no change in insulin sensitivity. Glucose oxidation in abdominal adipose tissue, however, was completely unresponsive to insulin stimulation after fasting. In conclusion, marked regional variations in fasting-mediated changes in insulin action on glucose metabolism at receptor- and post-receptor levels are present in human adipose tissue.

18. Is HLA-DR type or insulin formulation the major determinant of the antibody response to insulin?

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The insulin binding antibody (IAB) response to exogenous insulin has been studied prospectively in two groups of newly diagnosed HLA-DR typed childhood Type 1 (insulin-dependent) diabetic patients. Group A ($n = 53$) were randomly assigned to high purity pork insulin and group B ($n = 49$) to conventional beef/pork regular and isophane insulin. Follow-up blood samples were drawn at 1, 2, 4, 6, 9, and 12 months. All Type 1 diabetic patient developed IAB, but at all time points group B had higher IAB levels than group A irrespective of HLA-DR type. Thus, after 1 month insulin therapy those patients in group A who were DR3 only had IAB levels (mean ± SEM specific binding of tracer) of 3.9 ± 1.8%, those with DR4 had 4.6 ± 1.1% and those DR3/DR4; 4.6 ± 0.9%. In group B the respective values were 11.7 ± 4.1%, 16.8 ± 3.5% and 10.3 ± 1.1%. At 12 months, the group A values were 21.1 ± 4.1%, 17.7 ± 2.4% and 15.3 ± 1.7% and group B values were 43.6 ± 5.3%, 36.7 ± 4.4% and 27.6 ± 4.1% ($p < 0.01$ for all group A versus group B comparisons but differences DR3 versus DR4 versus DR3/DR4 within groups A and B were not significant). Thus, insulin formulation and not HLA-DR type is the major determinant of IAB formation in childhood Type 1 diabetes.

19. Improvement of drug prescription, including oral hypoglycaemic agents, by specific training of physicians

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Non-compliance, a well known cause of treatment failure, is usually blamed on the patient. This study was designed to determine whether a group of eight physicians, nearing completion of their hospital training, had acquired sufficient knowledge to inform their patients correctly when prescribing a new drug, in particular oral hypoglycaemic agents. The study was in three phases: (1) tape-recording of 24 consultations; (2) establishment with all physicians involved of the basic steps in drug prescription; (3) recording of 24 consultations 2 months later. During phase 2, five basic steps were identified: reformulation by the physician of the patient's complaints; diagnosis; treatment (expediency, modalities, side effects, evaluation by the patient, evaluation by the physician); reformulation of the prescription by the patient. Conclusions: In phase 1, the diagnosis was only stated explicitly in 30% of the consultations and two items were consistently absent (evaluation of treatment by the patient; reformulation of the prescription by the patient). Apart from expediency of treatment and side effects, frequency of all items was significantly increased in phase 3. In conclusion, physicians are partly responsible for poor patient compliance. Drug prescription by physicians can be readily improved by specific training.

20. Administration of cyclosporin A to recently-diagnosed Type 1 (insulin dependent) diabetic patients

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Twelve adult, recently-diagnosed (62 ± 10 days) Type 1-diabetic patients were treated with insulin + cyclosporin A (CyA) for 4–8 months and compared with 30 patients treated by insulin alone. Age, clinical duration of diabetes, HbA_{1c} values and initial insulin dosage were similar in both groups. The CyA dosage was adapted according to the drug levels in blood. A complete remission (tight control with < 4 U insulin/day) occurred and continues in 4 out of 12 CyA patients versus 3 out of 30 in controls. A partial remission (insulin requirement reduced by 50%) occurred in two further CyA patients. The mean insulin requirements dropped significantly from day 4 of CyA treatment and by day 120 were: 8 ± 3 versus 40 ± 5 U/day. The OKT4/OKT8 ratio, initially higher than normal in 9 out of 12, was rendered normal in five. Lymphocyte activation by phytohaemagglutinin and Interleukin 2 levels were suppressed. Anti-islet cellular and humoural immunity, initially detected in ten, were suppressed in three. No major difference in HLA and BF groups appeared between patients who did and those who did not remit. However initial ketoacidosis was more frequent and the basal and stimulated C-peptide levels were higher in those who remitted than in others. Minor side-effects (epigastric pain, hypertrichosis, gum swelling) were observed. Kidney and liver function remained normal.

21. Action of insulin in isolated hepatocytes of lean and obese rats

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In vivo, the liver of obese rats responds very poorly to insulin since high doses of the hormone are needed to decrease hepatic glucose production. To assess whether this represents an intrinsic defect of the hepatic cell to respond to insulin, isolated hepatocytes of fed lean and obese (fa/fa) rats were incubated and the glucagon-counteracting action of insulin was tested on phosphorylase *a* activity, glucose and lactate plus pyruvate output, and fructose 2–6 bisphosphate levels. Basal production of lactate plus pyruvate and level of fructose 2–6 bisphosphate were higher in hepatocytes of obese rats suggesting a higher rate of glycolysis. The data showed that, in hepatocytes of normal as well as obese rats incubated with 10^{-10} mol/l glucagon, insulin promoted: (a) a decrease in phosphorylase *a* activity; (b) a marked decrease in glucose production; (c) an increase in glycolytic products and fructose 2–6 bisphosphate. The concentrations of insulin needed for these actions were comparable in hepatocytes of lean and obese rats (100–250 mU/l). It is suggested that the hepatocytes of fed obese rats have no intrinsic defect in their capacity to respond to insulin and that the hepatic insulin resistance in vivo may be due to factors which disappear during the preparation of hepatocytes.

22. Seven cases of acanthosis nigricans with insulin resistance: new clinical presentations and the results of euglycaemic clamp studies

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The syndrome of acanthosis nigricans (AN), polycystic ovarian disease and insulin resistance (IR) is a well-established but rare disorder. Over 2 years seven cases of AN with IR have been studied. In some cases the presentation has varied from that described above. One was a man who presented with obesity, TSH and gonadotropin deficiency and an empty sella. His sister presented with oligomenorrhoea and hyperprolactinaemia. Another patient had been investigated for short stature (final height 137 cm) prior to presentation with oligomenorrhoea and diabetes. Growth hormone response to insulin-induced hypoglycaemia was normal (32 mU/l) and no cause for short stature other than IR has been discovered. Another patient presented with childhood dermatomyositis, calcinosis cutis and hirsutism. A 75 g oral glucose tolerance test in the group showed normal glucose tolerance in two patients, impaired tolerance in one and diabetes in four. Mean fasting serum insulin was 65 ± 11 mU/l (SEM) rising to a peak of 208 ± 28 mU/l. Corresponding plasma glucose levels were 8.1 ± 1.6 and 13.5 ± 2.3 mmol/l. Euglycaemic clamp studies were performed at similar fasting glucose levels in each case. Although all patients were shown to have IR, M values varied markedly from 0.31 to 2.92 $mg \cdot kg^{-1} \cdot min^{-1}$ at an insulin infusion rate of 40 mU/m^2 per min. The spectrum of presentations together with the clamp study results suggests that AN with IR may be underdiagnosed and that it may encompass a variety of endocrine defects.

23. Effect of exercise on protein metabolism of soleus muscles of lean mice and mice with obesity induced by goldthioglucose

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Among the various factors which influence protein metabolism in muscle, exercise plays a major role. Since physical exercise is considered beneficial in the treatment of obesity and diabetes, we have investigated the effect of 'pure' exercise on protein metabolism in muscles of lean and obese mice. Work-induced hypertrophy of soleus was obtained in one leg by severing the tendon of the gastrocnemius 4 days before experiment, muscle in the other leg being used as control. Tyrosine release and tyrosine incorporation into material precipitable by trichloroacetic acid (indices of protein degradation and synthesis respectively) were enhanced 2.5-fold in exercised muscles of lean mice and 1.8-fold in muscles of obese mice. The transport of an alanine analogue, α-aminoisobutyric acid, was enhanced 2.5-fold in exercised muscles. To localise the site of action of exercise in protein synthesis, initiation and elongation steps were studied: exercise increased 2.5-fold the number of nascent polypeptide chains, whereas elongation rates were unchanged. In conclusion, selective exercise, restricted to one muscle, increases protein turnover and suppresses the defect in amino-acid transport observed in muscles of obese mice. Exercise per se thus appears to have beneficial effects directly at the tissue level during obesity.

24. Glucose and ketone body turnover during continuous insulin infusion treatment in Type 1 (insulin-dependent) diabetic patients via subcutaneous and peritoneal routes

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Glucose concentration (G), glucose turnover (GT), clearance rate (GPCR), mean residence time (MRT), acetoacetate (AcAc) blood concentration, production (R_pAcAc), disposal (R_dAcAc) and 3-hydroxybutyrate (BOH) blood concentration, production (R_pBOH), disposal (R_dBOH) were studied in six normal subjects (N); in diabetic patients on continuous subcutaneous insulin infusion (12 ± 4 months): five diabetic patients with fixed basal (CSII-FBR); four diabetic patients with higher overnight infusion rate (CSII-HOR) and in four diabetic patients on continuous intraperitoneal insulin infusion (CPII) 1 ± 0.2 months). G was 4.01 ± 0.17 mmol/l in N, 5.42 ± 0.73 in CSII-FBR, 3.17 ± 0.39 ($p < 0.05$) in CSII-HOR, 4.0 ± 0.23 in CPII. No differences were observed in GT between normal and diabetic subjects. GPCR was 0.024 ± 0.003 ml$\cdot$kg$^{-1}\cdot$min^{-1} in N; 0.017 ± 0.002 in CSII-FBR ($p < 0.05$); 0.019 ± 0.002 in CSII-HOR and 0.017 ± 0.002 in CPII ($p < 0.05$). MRT was higher in diabetic than in normal subjects ($p < 0.05$). There were no differences in AcAc and BOH between both groups. R_pAcAc (μmol$\cdot$kg$^{-1}\cdot$min^{-1}) was higher in CSII-FBR (4.10 ± 0.44) than in normal subjects (2.16 ± 0.37) ($p < 0.05$) but not in CSII-HOR and in CPII. R_pBOH was higher in CSII-FBR than in N (9.14 ± 1.69 versus 2.85 ± 0.42, $p < 0.01$) and lower in CPII (0.39 ± 0.31, $p < 0.05$) but not in CSII-HOR. R_dAcAc was higher in CSII-HOR ($p < 0.01$) (8.99 ± 1.56) than in N. Free insulin was higher in diabetic than in normal subjects irrespective of the type of treatment. In conclusion, CSII-FBR achieves abnormal GT and ketone body turnover even if blood G and ketone bodies are normal. CSII-HOR exposes diabetic patients to the risk of hypoglycaemia. CPII achieves near normal G and ketone body turnover but not normal insulin.

25. Carbohydrate metabolism in normal human pregnancy: possible alteration in hepatic extraction of insulin

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Little is known about the hepatic extraction of insulin in human pregnancy. We have investigated this by measuring the concentration of plasma glucose (PG), IRI and C-peptide in post-absorptive venous blood samples obtained serially at <12, 16, and 32 weeks gestation and 6 weeks after delivery from 86 randomly selected normal pregnant women (P), and on one occasion from 90 normal, age-matched, non-pregnant women (NP). All data for both groups were normally distributed. There was a small but significant rise in plasma glucose up to 32 weeks gestation (mean $\pm$ SEM for NP 3.86 ± 0.06; P 3.99 ± 0.06, 4.17 ± 0.07, 4.33 ± 0.14), and post-partum pregnant women (PP) (4.23 ± 0.08 mmol/l; p NS, <0.001, 0.01, 0.001 for NP versus P and PP). IRI also increased (NP 0.08 ± 0.005; P 0.10 ± 0.008, 0.14 ± 0.01, 0.14 ± 0.005; PP 0.08 ± 0.006 nmol/l; $p < 0.05$, 0.001, 0.001, NS), with a significant rise in $\frac{IRI}{PG}$ ratio at 16 and 32 weeks gestation ($p < 0.001$, 0.001). In contrast there was no rise in C-peptide until 32 weeks gestation (NP 0.65 ± 0.03; P 0.57 ± 0.04, 0.52 ± 0.03, 1.52 ± 0.16; PP 0.69 ± 0.04 nmol/l; p NS, NS, <0.001, NS). Despite this, the $\frac{CP}{IRI}$ molar ratio was significantly lower throughout (NP 8.99 ± 0.39, P 6.09 ± 0.30, 5.47 ± 0.48, 7.60 ± 0.35; $p < 0.001$, 0.001, 0.01). Mean fasting $\frac{CP}{IRI}$ ratio in 29 third trimester P was 7.9 ± 0.47, i.e. NS different from post-absorptive values. Results suggest that reduced hepatic extraction of insulin may contribute significantly to the peripheral hyperinsulinaemia of pregnancy.

26. Intestinal mucosa preferentially releases somatostatin-28 in pigs

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Immunoreactive somatostatin (SLI) consists mainly of two forms in plasma, corresponding to somatostatin (SS) 1–14 and 1–28; 46% of plasma SLI is due to the SS-28-like component in man, but its source is unknown. We therefore studied the molecular nature of newly released SLI from isolated, perfused, porcine pancreas, stomach, antrum and small intestine. The organs were perfused with synthetic medium in a single pass system, and SLI secretion from stomach, antrum and small intestine (a 25 cm segment of ileum or jejunum) was stimulated by intraluminal introduction of 20 ml 0.1 mol/l HCl. Effluent samples were concentrated by adsorption on to Sep-pak C-18 cartridges and SLI eluted using 96% EtoH with 1% trifluoroacetic acid. The dessicated eluates were subjected to Sephadex G 50 gel filtration and radioimmunoassays against the 5–10 sequence of SS-14 as well as the N-terminal part of SS-28. More than 95% of newly secreted SLI from the pancreas co-chromatographed with SS-14, whereas 68.07 ± 1.66 and 64.47 ± 2.92% of the jejunal and ileal SLI, respectively, co-chromatographed with SS-28. Peak fractions from all tissues studied co-chromatographed with SS-14 and SS-28 standards on high performance liquid chromatography. In conclusion, the upper intestinal mucosa, rather than the pancreas, is the likely source of SS-28 in the circulation.

27. Changes in gastrointestinal substance P, vasoactive intestinal polypeptide and somatostatin following streptozotocin-induced diabetes

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The effect of streptozotocin-induced diabetes upon the content of substance P, vasoactive intestinal polypeptide (VIP) and somatostatin in the rat gastrointestinal tract was investigated. To identify non-specific effects of streptozotocin, diabetic rats injected with streptozotocin (65 mg/kg; group A; n = 14) were compared with non-diabetic rats, injected with streptozotocin (65 mg/kg) + nicotinamide (500 mg/kg; group B; n = 8) and injected with saline only (group C; n = 8). Rats were sacrificed after 10 weeks. The stomach weight in the three groups was not significantly different, but the small intestine weight in group A was increased (167% versus group B, 165% versus group C; $p < 0.001$). In the intestine of group A rats, total content of substance P was lower (68%, $p < 0.01$), VIP higher (154%, $p < 0.05$) and somatostatin lower (64%, $p < 0.01$) than in group B rats. In the stomach of group A rats, substance P content was less (63%, $p < 0.01$) than in group B, but content of VIP and somatostatin were not significantly different. Differences in tissue content of substance P and VIP in group B rats were not different from group C, but the gastric somatostatin content of group B was greater (158%, $p < 0.05$) than in group C. In conclusion, changes in the content of substance P and VIP in gastrointestinal tissues follow streptozotocin-diabetes, but changes in somatostatin may represent a non-specific effect of streptozotocin rather than of diabetes.

28. Placental glycogen accumulation in hyperglycaemic non-diabetic rats

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Streptozotocin-diabetes in pregnant rats has been shown to induce glycogen accumulation in the placenta. To explore the mechanism of this accumulation, rats on day 19.5 of gestation received an intravenous glucose infusion for 48 h (300 mg$\cdot$h$^{-1}\cdot$kg^{-1}). On day 21.5 maternal serum glucose was 25 ± 3 mmol/l and insulin 224 ± 34 mU/l versus 5.0 ± 0.5 mmol/l and 125 ± 15 mU/l in control rats similarly infused with glucose (30 mg$\cdot$h$^{-1}\cdot$kg^{-1}). Fetal serum glucose was 15.0 ± 1.0 versus 1.9 ± 0.2 mmol/l, respectively. Placental glycogen

content in hyperglycaemic rats was 5.2 ± 0.7 versus 1.8 ± 0.3 mg/g in control animals ($p < 0.001$). Maternal liver glycogen was also significantly increased (65.5 ± 6.2 versus 30.9 ± 8.2 mg/g), whereas fetal liver glycogen did not change appreciably (80.6 ± 10.3 versus 86.9 ± 7.7 mg/g, respectively). The placenta was separated into the decidual part and the spongious fetal villi. Glycogen distribution in hyperglycaemic rats was 6.8 ± 0.5 and 3.5 ± 0.5 mg/g correspondingly, whereas in control rats the glycogen content was 3.6 ± 0.6 and 1.4 ± 0.4 mg/g in the two parts of the placenta. Thus, in the fetoplacental unit the glycogen rose only in the placenta as a result of maternal hyperglycaemia. Since a similar rise in placental glycogen occurs in diabetic, insulin-deficient rats, we conclude that the determining factor for placental glycogen accumulation is the abundance of substrate rather than insulin stimulation of glycogen synthesis.

29. Low plasma C_4 concentrations: association with microangiopathy in insulin-dependent diabetes

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During investigations of immune mechanisms in the aetiology of microangiopathy, we have measured plasma C_4 concentrations in 72 Type 1 (insulin-dependent) diabetic patients without complications, 17 with severe and 8 with mild microangiopathy and 57 control subjects. In 41 of the diabetic patients the albumin excretion rate was measured. The diabetic patients had a significantly lower distribution of C_4 values than the control subjects ($p < 0.005$) and diabetic patients with complications had a lower distribution than those without ($p < 0.025$); the respective median values were also significantly lower ($p < 0.001$). Of 26 subjects with low C_4 (≤ 0.23 g/l), 14 had microvascular disease – ten severe and four mild. The relative risk for development of severe or any microvascular complications for subjects with low C_4 was increased 7.1 and 6.3 times, respectively. Of the 72 diabetic patients without complications, 12 had low C_4, but nine had only been diagnosed within the last 7 years – three of five tested showed increased albumin excretion rate. Of 13 subjects with increased albumin excretion rate (range 21–190 μg/min), 11 had low C_4. There was no evidence of complement activation. Low plasma C_4 concentrations in Type 1 diabetic patients appear to be strongly associated with microangiopathy.

30. Normalization of renal function in Type 1 (insulin-dependent) diabetes induced by improved metabolic control for 1 year

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The effect of strict metabolic control on renal function was estimated in 24 Type 1 (insulin-dependent) diabetic patients (age 27.5 ± 7.5 years, duration of diabetes 10.2 ± 5.8 years) with Albustix-negative urine randomized to conventional insulin treatment (CIT) and to continuous subcutaneous insulin infusion (CSII) with a portable pump. After 1 year of treatment we found in patients with improved metabolic control a statistically significant normalization of both the glomerular filtration rates (GFR) (136 ± 14 to 120 ± 15 ml/min, $p < 0.01$) and the urinary albumin excretion (22.5 ± 23.0 to 9.8 ± 4.3 mg/24 h, $p < 0.05$). On the other hand, in the group of impaired or unchanged control we found no changes in GFR values (122 ± 18 to 123 ± 13 ml/min, $p < 0.1$) whereas the albumin excretion increased (9.5 ± 10.00 to 12.3 ± 9.4 mg/24 h, $p < 0.01$). Furthermore, in the total material before treatment we found a positive correlation between the metabolic status, estimated by the HbA_{1c} values, and GFR as well as albumin excretion ($r = 0.64$, $p < 0.01$ and $r = 0.42$, $p < 0.05$). Thus, we conclude that extensive elevation of GFR values and slightly increased urinary albumin excretion found in Type 1 diabetic patients after 10 years of conventional treatment can be rendered normal by near-normalization of metabolic control for 1 year.

31. Evidence for heterogeneity in the latency period of Type 1 (insulin-dependent) diabetes

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There is considerable evidence of heterogeneity within Type I diabetes, with a sub-group characterized by later onset, female preponderance, co-existence of autoimmune endocrine disease and persistence of islet-cell antibodies (ICA). We screened a large population referred for endocrine investigation and identified 173 ICA-positive non-diabetic individuals, six of whom have already developed diabetes. The remaining patients are now taking part in a prospective study which will allow comparison with the Barts-Windsor-Middlesex-family study in terms of serological, immunological and genetic characteristics. Initial comparison with 58 ICA-positive, non-diabetic siblings and parents in the family study emphasizes the different nature of the two populations. The incidence of organ-specific (i. e. thyroid, gastric, adrenal) and non-organ specific (i. e. ANA, ENA, mitochondrial) antibodies in this new group is 3–5 times that found in the family study population, even when similar age groups are compared. The mean age of initial ICA-detection (43.1 versus 25.1 years) and the female/male ratio (2.5 versus 0.7) are considerably higher in this new group of patients, compared with the relatives in the family study. The percentage of complement-fixing ICA reaches 66% in the new group, compared to 24% in the non-diabetic family study group. This analysis confirms the existence of a distinct sub-group of patients in which Type I diabetes forms part of an autoimmune polyendocrine syndrome.

32. Analysis of QT versus RR ECG interval variations in diabetic patients shows a longer QT period in subjects with autonomic neuropathy

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QT versus RR (QTc) ECG interval variations were analyzed in 24 male Type 1 (insulin-dependent) diabetic patients (aged 32 ± 6 years; mean ± SD) previously divided – 8 without, 7 borderline, 9 with diabetic autonomic neuropathy (DAN) using a scoring-system based on cardiovascular response to Valsalva manoeuvre (VM), deep-breathing, lying-to-standing ratio and sustained handgrip. Results were compared with those of 13 healthy sex and age-matched control subjects. In DAN subjects, QTc resting values were 385 ± 14 ms lying, 413 ± 20 ms sitting and 397 ± 16 ms standing, significantly higher than in controls (lying: 365 ± 19 ms, $p < 0.05$; sitting: 382 ± 10 ms, $p < 0.01$; standing: 376 ± 13 ms, $p < 0.01$) and in diabetic subjects without DAN (lying: 352 ± 40 ms, $p < 0.05$; sitting: 383 ± 83 ms, $p < 0.05$; standing: 379 ± 14 ms, $p < 0.05$). Further analysis of QT variations under various dynamic conditions, namely VM and standing-to-lying (SL) revealed similar QTc values in all groups examined only during induced maximal tachicardia, while, during induced maximal bradicardia, QTc values were markedly higher in DAN subjects (VM: 415 ± 20 ms; SL: 388 ± 14 ms) than in controls (VM: 346 ± 20 ms, $p < 0.001$; SL: 360 ± 32, $p < 0.05$) and in diabetic subjects without DAN (VM: 344 ± 20, $p < 0.001$; SL: 356 ± 30, $p < 0.02$). As a different approach using QT measured (QTm) gives similar results, it can be concluded that QT period under resting conditions is longer in autonomic patients and such patients have a QT versus RR relationship on resting similar to that found in non-autonomic subjects under stress-induced tachicardia.

33. Gastric inhibitory polypeptide response to oral glucose in gestational diabetes

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A diminished incretin effect has been observed in gestational diabetes. Further investigation is necessary since only a few patients have been studied and since the aetiology of gestational diabetes appears to be so heterogeneous. The aim of this study was (1) to measure the response of gastric inhibitory polypeptide (GIP) following oral glucose in late pregnancy, (2) to investigate the reproducibility of GIP secretion under these circumstances, and (3) to examine the possible correlation between GIP secretion and insulin output in gestational diabetes. 80 young women of whom 60 were in the third trimester of pregnancy, received an oral glucose load (100 g). Venous blood samples were taken at 15-min intervals over 3 h and analysed for glucose, insulin, C-peptide, and GIP levels. 20 non-pregnant and 20 pregnant women were tested twice within one week. The glucose-stimulated GIP secretion was markedly diminished in pregnancy as compared with the non-pregnant state. The GIP profiles were much more reproducible than the insulin curves. In contrast to insulin, the GIP levels did not differ significantly between normal pregnant women and the 30 gestational diabetics. It is concluded that GIP secretion is impaired in late pregnancy, and that in gestational diabetes abnormal insulin secretion and GIP response to oral glucose are not related.

34. Intermittent clinical proteinuria and renal function in diabetes: evolution and the effect of glycaemic control

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We report a preliminary study of the evolution of renal failure in 12 intermittently proteinuric insulin-dependent diabetic patients (1 female; 11 males; mean age 38.5 ± 11.2 years; duration of diabetes 22.8 ± 5.7 years). After a mean 16 month run-in period, age- and duration-matched patients were allocated to either continuous subcutaneous insulin infusion (CSII) or conventional injection therapy (CIT) and studied for a further 15 months. Mean plasma glucose improved significantly in the CSII group (8.8 ± 2.0 versus 6.2 ± 2.4 mmol/l; $p < 0.01$, run-in versus experimental periods) but not in the CIT group. Blood pressure was maintained normal throughout the study. Glomerular filtration rate (GFR) fell significantly in both groups throughout the study from baseline values of 104.3 ± 25 and 118.7 ± 16 ml/min per 1.73 m^2 to final values of 77.8 ± 26.4 and 94.5 ± 22.5 ml/min per 1.73 m^2 (CSII and CIT respectively). The mean rate of GFR decline did not change significantly in either group (run-in versus experimental period, CSII: 0.6 versus 0.5 ml/min per month; CIT: 0.9 versus 0.9 ml/min per month). Plasma creatinine rose slightly in the CSII group only (CSII 98 ± 10 to 114 ± 27; CIT 90 ± 15 to 86 ± 11 μmol/l). The fractional clearance on albumin varied unpredictably. Thus (1) glomerular function is already deteriorating progressively at the stage of intermittent proteinuria; (2) no significant effect was observed during glycaemic control.

35. Effects of the composition of mobile phase on the immunochemical properties of A_{14}-^{125}I-insulin purified by high performance liquid chromatography

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High performance liquid chromatography (HPLC) allows the separation of ^{125}I-iodoinsulin selectively labelled on one of the four available tyrosines. The effects of the eluent composition on the immunochemical properties of A_{14}-^{125}I-insulin (A_{14}-Ins) were studied after purification of the isomer by HPLC using various eluents: (a) phosphate buffer 0.01 mol/l, acetonitril, isopropanol, NH_4OA_c 0.15 mol/l; (b) phosphate buffer 0.01 mol/l, acetonitril, isopropanol, $NaClO_4$ 0.1 mol/l; (c) acetonitril, trifluoracetic acid 0.1% in water. The results were compared with those obtained using A_{14}-Ins separated by PAGE. Maximum binding to antiserum was similar for A_{14}-Ins purified by PAGE and by HPLC using eluent A (93 ± 6% and 94 ± 4% respectively) and lower for A_{14}-Ins purified by HPLC using eluent B (87 ± 8%) and C (85 ± 8%). The antibody dilution curves showed the binder dilutions able to bind 50% of the tracer were 1:335,000 ± 49,000, 1:353,000 ± 61,000, 1:200,000 ± 69,000 and 1:182,000 ± 71,000 for A_{14}-Ins, obtained by PAGE and by HPLC with eluents A, B, C, respectively. The K values computed at high and low affinity antibody receptor sites were similar for A_{14}-Ins by PAGE and HPLC, eluent A and lower for A_{14}-Ins chromatographed with eluents B and C. In conclusion, important differences exist among A_{14}-Ins obtained by HPLC using various eluents; A_{14}-Ins by HPLC, eluent A and PAGE show similar immunochemical properties.

36. Effects of Ca^{2+} and glucose on multiplication and functional differentiation of clonal insulin-producing cells (RINm5F)

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The significance of RINm5F cells for exploring the insulin-releasing mechanism is critically dependent on the extent to which these cells retain the characteristics of normal β cells. The composition of the culture medium is one of several factors which may modify the specific function of continously growing cell lines. The RINm5F cells were therefore investigated with regard to effects of extracellular Ca^{2+} and glucose on proliferation and functional differentiation. The proliferation and release of insulin were equally effective at concentrations of Ca^{2+} ranging from 0.16 to 4.4 mmol/l. In contrast to normal β cells the amounts of calcium in the RINm5F cells remained relatively constant after lowering the extracellular concentration of Ca^{2+} and inhibiting the entrance of the ion with D-600. Culture at 4.4 mmol/l Ca^{2+} resulted in substantial incorporation of cellular calcium and a simultaneous reduction of the insulin content. Cells deprived of glucose had a significantly lower dry weight, were less able to exclude trypan blue and contained and released less insulin. Since protein biosynthesis is strictly dependent upon an adequate intracellular concentration of Mg^{2+}, these long-term effects of glucose removal may be related to the concomitant decrease in the cellular content of magnesium.

37. Early diagnosis of diabetic cardiac autonomic damage by on-line computer analysis of heart rate-respiration relationship: preliminary study

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This study compares the sensitivity of electrocardiographic R-R methods, used alone and with respiration, for diagnosis of cardiac autonomic dysfunction in diabetic patients. In 16 normal subjects (aged 43 ± 12 years; group A), 17 diabetic patients without neuropathy (aged 44 ± 13 years; group B) and 11 diabetic patients with autonomic neuropathy (aged 45 ± 11 years; group C), ECG and respiration during normal breathing and deep breathing at 4, 6 and 18 breaths/min were recorded 'on-line' and fed into an Apple II computer. Heart rate, R-R standard deviation (SD), R-R range and cross-correlation function (complex product of R-R and respiration Fourier transforms, transformed back in time domain) (CC) were computed. During normal breathing, heart rate was 71.6 ± 14.9 beat/min in group A, 76.6 ± 11 in group B, and 87.3 ± 12.4 in group C ($p < 0.05$); R-R SD was 49.2 ± 26.5 ms in group A, 29.3 ± 13.6 in group B and 16.6 ± 9.0 in group C ($p < 0.001$); R-R range was 272 ± 167 ms in group A, 169 ± 75 in group B and 101 ± 51 in group C ($p < 0.005$); CC was 3.8 ± 0.5 in group A, 3.0 ± 0.7 in group B and 2.3 ± 0.6 in group C ($p < 0.0001$). During deep breathing the difference between groups A and B increased for R-R SD, R-R range ($p < 0.01$) and CC ($p < 0.001$). CC better discriminates between normal and diabetic subjects without neuropathy during normal breathing; deep breathing enhances the relationship between heart rate-respiration in normal subjects and to a lesser degree in diabetic patients.

38. How accurate are insulin mixtures prepared by the patient?

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The accuracy of delivery of the two components of a mixture prepared in one insulin syringe was examined in 41 insulin-treated diabetic patients, using both a syringe with a separate needle and one with a fixed needle (small dead space). The composition of a mixture of 0.9% NaCl and water was evaluated by conductivity measurements for the following proportions: 0.3/0.3 ml, 0.2/0.4 ml and 0.1/0.5 ml. Syringes with a separate needle delivered an overdose of 0.048–0.067 ml of the 'first insulin' (NaCl), 112%, 124% and 167% of the intended dose, corresponding to 5–7 units of U-100 insulin. Insulin accommodated in the dead space caused an insulin wastage of 9 units (U-100), compared with 1 unit in syringes with a small dead space, which delivered insulin in mixtures within ±2 units of the intended dose. Contamination of the 'second insulin' vial by the 'first insulin' was negligible. Clinically important dosage errors are likely for patients, who, according to self-monitoring of glucose, adjust the dose of individual components of insulin mixtures prepared in syringes with separate needles, particularly when U-100 insulin is used. Therefore syringes with fixed needle should be preferred for the preparation of insulin mixtures.

39. Thermal discrimination thresholds in patients with diabetic neuropathy

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Quantitation of cutaneous sensation is important when evaluation of therapy in patients with diabetic neuropathy is required. We used a method to determine the ability of human subjects to discriminate between different temperatures at the skin. By means of the Peltier principle, two identical plates can be set and maintained at different temperatures. Plate 1 was maintained at a constant temperature which equalled the skin temperature of the test area. The temperature of plate 2 was varied and was randomly adjusted higher or lower than the temperature of plate 1. At each step, the difference in temperature between the two plates was reduced and by means of a two-alternative, forced-choice procedure, the thermal discrimination threshold was determined. In 30 normal subjects under 70 years of age, the threshold for the foot was between 0.15 °C and 0.50 °C. In 15 diabetic patients without symptoms of neuropathy, discrimination thresholds ranged from 0.15 °C to 2.50 °C. In 15 diabetic patients with painful neuropathy, values varied from 0.70 °C to > 10.0 °C. It is concluded that determination of thermal discrimination thresholds is a simple and reliable method for quantitating sensory disturbances in patients with diabetic neuropathy.

40. Short-term insulinopenia induces insulin resistance in normal men

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Both hyperinsulinaemia and hypoinsulinaemia are encountered in clinical conditions of insulin resistance. A down-regulation of insulin receptors, as well as regulation of post-receptors events, may play a rôle in the genesis of insulin resistance. The present study was aimed to evaluate the rôle of short-term insulinopenia on insulin receptors and insulin sensitivity. Eleven normal men were studied (aged 20–26 years, non-obese and with no family history of diabetes). Insulinopenia was induced by a 5 h somatostatin infusion (250 μg/h). Insulin receptors on circulating monocytes were evaluated before and after 4 h insulinopenia; in five of the subjects an intravenous insulin tolerance test (0.05 mU/kg) was performed after a 4 h saline or somatostatin infusion. Insulin binding showed irregular changes after the somatostatin infusion (2.19 ± 0.30 before and 2.35 ± 0.80 after) while insulin sensitivity was decreased after 4 h of insulinopenia in all five subjects (K_{ITT} 1.0 ± 0.3 after somatostatin, 3.8 ± 0.4 after saline; $p < 0.001$). No relationship was found between insulin sensitivity and insulin binding. The present data demonstrate that short-term insulinopenia induces a condition of insulin resistance; insulin receptors do not seem to be involved, thus suggesting that an acquired post-receptor defect may play a major rôle in the genesis of insulin resistance.

41. Association between a rare allele of the second component of complement (C2*2) and Type 1 (insulin-dependent) diabetes in families with additional DR3- and/or DR4- associated diseases

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Major histocompatibility complex (MHC) markers (HLA, C2, C4, Bf) were determined in 26 families with Type 1 diabetes and other DR3- and/or DR4-associated diseases, such as rheumatoid arthritis, Graves' disease or coeliac disease. Results were compared with analogous values from single-case diabetic families. A highly significant increase of the rare C2*2 allele of the second complement component was found ($p < 0.005$). The rare alleles of the factor B (BF) polymorphism (BF*F1, BF*S0.7) and of the fourth component of complement (C4*B2, C4*B3) also showed increased frequencies. These data suggest that within diabetic families rare alleles of the class III MHC genes besides DR3 and DR4 may contribute to the development of additional DR3- and DR4-associated diseases.

42. Portal and peripheral administration of insulin in diabetic dogs: effects of the artificial β-cell on turnover rates of glucose and alanine

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Long-term diabetic dogs (no canine C-peptide, no insulin antibodies) with chronic portal venous catheters were treated for 10 h with an artificial β cell administering insulin either by the portal or the peripheral route. After restoration of normoglycaemia, the turnover of glucose (6-^{3}H-glucose) and alanine (U-^{14}C-alanine) and gluconeogenesis from alanine were investigated under basal conditions and during an intravenous glucose load. Glycaemia and glucose turnover under basal conditions were entirely normal, independent of the route of insulin administration. During and after glucose load, however, glycaemia was slightly elevated on the basis of a lower rate of glucose disappearance when portal insulin was given (dose higher but plasma insulin lower than by peripheral infusion). Concentration, turnover rates of alanine and gluconeogenesis from it were also higher by portal insulin. It is concluded that the metabolism of glucose and its precursors were appreciably influenced by the route of insulin administration. Only portal insulin prevents peripheral hyperinsulinaemia. The higher alanine turnover is based on a primary elevation of alanine production which is fully taken up by the normally insulinized liver. Both long-term observations and exteral administration of the glucose load are needed to elucidate the importance of the portal route.

43. A second messenger function of inositol-1,4,5 trisphosphate in insulin secretion

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Inositol-1,4,5 trisphosphate (IP_3) has been recently attributed second messenger function because it is rapidly generated during cell activation and mobilizes intracellular Ca^{2+}. We examined the possible role of IP_3 in insulin secretion by using Ca^{2+} specific electrodes to monitor the steady state Ca^{2+} level maintained by digitonin-treated RINm5F cells. Only intracellular Ca^{2+} buffering mechanisms operate in this system because the plasma membrane is permeabilized. In a medium mimicking cytosolic ionic composition the cells maintained a Ca^{2+} steady state of 119 nmol/l, almost identical to cytosolic Ca^{2+} concentrations reported for intact RIN cells. Maintenance of this level required Ca^{2+} uptake into a non-mitochondrial ATP-dependent, vanadate inhibitable pool, tentatively identified as endoplasmic reticulum. IP_3 promoted rapid Ca^{2+} release specifically from this pool with apparent Km of ≈ 0.5 μmol/l. Maximal concentrations (2.5 μmol/l) released ≈ 10 nmol Ca^{2+}/mg cell protein, followed by a slower Ca^{2+} re-uptake. ^{32}P labelled IP_3 was degraded by this preparation. In separate experiments intact cells were isotopically equilibrated with [^{3}H]myo-inositol. 1 min exposure to the secretagogue D-glyceraldehyde, also known to raise cytosolic Ca^{2+}, promoted a significant increase in IP_3 radioactivity. Taken together the results suggest that IP_3, by mobilizing intracellular Ca^{2+}, may act as a second messenger linking metabolic and ionic events during nutrient stimulated insulin release.

44. Influence of the basal rate pulse interval on the pharmacokinetics of subcutaneously infused insulin

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To compare the pharmacokinetics of insulin infused using an auto-syringe pump providing a basal rate in pulses every 6 min with a Medix pump providing a basal rate in pulses every 60 min, eight insulin-dependent diabetic patients were given constant infusions of ^{125}I-labelled Actrapid HM insulin into the abdominal subcutaneous tissue for 12 h with these two pumps (1 IU/h in each side of the abdomen). The size of the subcutaneous depots was continuously measured externally by counting the radioactivity at the infusion sites. Subcutaneous depots were built up reaching steady-state levels at the same time and size (approximately 3 IU) and with similar absorption rates from both depots. In another study, six C-peptide-negative diabetic patients received identical insulin treatment during one 4-day period using the auto-syringe pump and another 4-day-period using the Medix pump. On day 4 of each period, blood glucose and plasma immunoreactive insulin were estimated ½-hourly for 7 h and every 5 min for a further 1 h. No differences in blood glucose or plasma insulin profiles were found in the two periods. In conclusion, during basal insulin infusion identical insulin absorption and appearance kinetics were achieved irrespective of a ten-fold difference in the pulse rate used.

45. Subcutaneous insulin absorption in lean and overweight men

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It is important to be aware of factors, other than the site of subcutaneous injection, that can influence insulin absorption. We have studied the disappearance of ^{125}I-labelled human Actrapid insulin in six overweight, otherwise normal male volunteers (115%–134% ideal body weight) for 6 h after subcutaneous injections into the abdomen. Simultaneous estimation of plasma immunoreactive insulin (IRI), C-peptide and glucose was conducted. Identical studies were performed in five lean male volunteers (85%–100% ideal body weight). The disappearance of ^{125}I-human Actrapid insulin was considerably faster in the lean subjects ($t_{½}$ disappearance 121.5 ± 38 min compared with 223 ± 52.9 min, $p < 0.01$). The more rapid absorption was confirmed by the incremental changes in plasma IRI, these being significantly higher in the lean group between 30 and 90 min after injection ($p < 0.02$). The increment in plasma IRI was significantly higher in the overweight group after 270 min until the end of the study, in keeping with the slower absorption pattern ($p < 0.02$). The plasma C-peptide and glucose results showed similar trends. It is important to consider the effects of increasing adiposity on insulin absorption, particularly from an abdominal injection site.

46. Glycation of human very low density lipoproteins

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Very low density lipoprotein (VLDL) abnormalities occur in diabetes, and may be due to apolipoprotein glycation. Total glucose incorporation into human VLDL and also incorporation through glucose-lysine links were investigated. VLDL (1–2 mg protein/ml), bovine serum al-

bumin (BSA) and glucose-free controls were incubated in 80 mmol/l glucose and phosphate (50 mmol/l, pH 7.4)-buffered NaCl at 37 °C for up to 14 days. Incorporation of glucose (and D[U-^{14}C]-glucose) into VLDL, subsequently precipitated by trichloracetic acid, was 22 and 112 nmol glucose/mg protein at 2 and 6 days. BSA values were 7.8 and 12 nmol/mg, respectively. Glycated VLDL was extensively dialysed, delipidated, and 100 µg protein incubated with 1 mmol/l $NaBH_4$ containing 1 µCi NaB^3H_4 for 2 h at 37 °C. BSA-carrier was added and trichloracetic acid-precipitated protein counted for ^{3}H. Lysine glycation (measured as $NaBH_4$ reducible material) was insignificant at 6 days, and 1.4 nmol/mg protein at 13 days, compared with 0.7 and 1.5 for BSA. Human VLDL can be glycated, and thus detected in small samples. Glucose-VLDL binding may occur by interactions other than glycation with lysine.

47. Effect of chronic administration of a long-acting somatostatin analogue on glucose homeostasis

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Somatostatin prevents insulin secretion and chronic administration would thus be expected to impair glucose homeostasis greatly. We have recently used a long-acting somatostatin analogue (SMS 201–995: Sandoz) in the chronic treatment of patients with metastatic symptomatic gut-hormone producing tumours. SMS was given subcutaneously in a dose of 50 µg twice a day. The patients were continued on a normal diet and fasting blood glucose was monitored before and after starting SMS treatment. Four patients (2 VIPomas, 1 GRFoma, 1 glucagonoma) have been treated with SMS for >2 months, 1 for 10 months and all have responded symptomatically with significant suppression of abnormal peptide secretion. The two VIPoma patients had fasting blood clucose levels in the normal range (4.0 to 5.5 mmol/l) prior to SMS therapy. This was maintained in one after starting treatment while, in the other, the fasting blood glucose level ranged between 5.6 and 5.8 mmol/l. The fasting blood glucose ranges in the GRFoma patient before and after SMS therapy were 6.5–6.9 and 6.4–7.4 mmol/l, respectively. Similarly, the ranges in the glucagonoma patients were 5.6 to 5.8 and 5.5 to 6.6 mmol/l, respectively. We conclude that chronic administration of pharmacological doses of somatostatin does not significantly impair glucose homeostasis, probably as a result of the marked inhibition of glucagon and growth hormone and considerable delay in post-prandial glucose absorption. The therapeutic possibilities of "resting the B cell" need investigation.

48. Optimal quality of blood glucose self-monitoring by unselected Type 1 (insulin dependent) diabetic patients following improvement of a training programme

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The quality of blood glucose self-monitoring (BGSM) using Chemstrip 20-800 (Boehringer Mannheim) was evaluated in 67 patients (mean age 35 years, mean duration of diabetes 8 years) consecutively admitted to our inpatient, Monday-to-Friday teaching and treatment programme. Results of BGSM assessed on Tuesday mornings (following initial training on Monday afternoon) were compared with simultaneous laboratory measurements: correlation coefficient $r = 0.78$, mean difference between BGSM and laboratory $d = 1.89$ mmol/l. After the standard training, on Friday mornings, the quality of BGSM was insufficiently improved when compared with the respective Tuesdays' results: $r = 0.83$, $d = 1.45$ mmol/l, $p < 0.05$. Thus the training was intensified and re-evaluated in another 101 patients (age 33 years, duration of diabetes 9 years). The intensified programme led to optimal quality of BGSM: $r = 0.96$, $d = 0.78$ mmol/l (Friday) versus $r = 0.74$, $d = 2.28$ mmol/l (Tuesday) ($p < 0.001$). Furthermore, 57 unselected patients showed excellent quality of BGSM 14 ± 2 (mean ± SD) months after the initial training: $r = 0.95$, $d = 0.99$ mmol/l. These data demonstrate the importance of quality control of BGSM in the context of the evaluation of diabetes teaching programmes. It was shown that with intensified teaching procedures unselected Type 1 diabetic patients can achieve optimal quality of BGSM using a visual method (without a reflectance meter) only.

49. Effect of oral and intravenous glucose on the short-term regulation of insulin action in human fat cells

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The effects of various forms of glucose administration on insulin action in adipocytes were investigated in 26 female subjects. Subcutaneous adipose tissue was obtained before and (a) 60 min after oral glucose ingestion, (b) after a 60 min intravenous (IV) glucose infusion and (c) at 30 and 60 min after an IV glucose load. The high-affinity adipocyte insulin binding was 30% decreased after IV glucose ($p < 0.01$), whereas after oral glucose it was unaltered. Following oral glucose the sensitivity to the anti-lipolytic effect of insulin was enhanced 10-fold ($p < 0.01$). Insulin sensitivity after IV glucose was unchanged. Plasma glycerol was more markedly lowered after oral than after IV glucose. In conclusion, orally, but not IV administered glucose, mediates a rapid increase in insulin sensitivity in human fat cells. The lack of coherence between changes in insulin binding and sensitivity suggests that, following glucose administration, separate short-term regulatory mechanisms are operating at receptor and post-receptor levels of insulin action.

50. Insulin resistance in Type 1 (insulin-dependent) diabetes: roles of loss of β-cell function, peripheral hyperinsulinaemia and abnormal glycaemic control

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To assess the factors responsible for insulin resistance in Type 1 diabetes, 12 patients on long-term intensive insulin therapy were studied. Seven had undetectable C-peptide, 0.68 ± 0.04 U·kg^{-1}·day^{-1} insulin requirement with a mean blood glucose of 7.4 ± 0.5 mmol/l (group A) whereas five had residual β-cell function (≈50% C-peptide), 0.22 ± 0.03 U·kg^{-1}·day^{-1} insulin dose with a mean blood glucose of 5.8 ± 0.03 mmol/l (group B). Insulin dose-response curves for suppression of glucose production (GP) and stimulation of glucose utilization (GU) were determined (3-^{3}H-glucose), clamping plasma glucose at 4.7 ± 0.2 mmol/l and infusing insulin at 0.2, 0.28, 0.4, 10 mU·kg^{-1}·min^{-1} (sequential steps, 3 h each), somatostatin (0.25 mg/h) and glucagon (0.65 ng·kg^{-1}·min^{-1}). Plasma free insulin concentration required for half-maximal suppression of GP and stimulation of GU were greater in group A (22 ± 1 and 47 ± 3 mU/l) than in group B (16 ± 0.9 and 31 ± 2 mU/l, $p < 0.01$) and eight non diabetic control subjects (15.5 ± 0.7 and 33 ± 2 mU/l, $p < 0.01$). Maximal GU in group A (50 ± 1 µmol·kg^{-1}·min^{-1}) was lower than in group B (72 ± 2 µmol·kg^{-1}·min^{-1}) and normal subjects (75 ± 3 µmol·kg^{-1}·min^{-1}; $p < 0.01$) indicating an overall defect in glucose transport. In conclusion, in Type 1 diabetes, insulin resistance develops to a similar extent in both hepatic and extrahepatic tissues, only subsequent to total loss of β-cell function. Insulin resistance does not appear to be reversed by intensive insulin therapy since peripherally delivered insulin fails to normalize glycaemic control fully without hyperinsulinaemia.

51. Development of diabetes in the BB/E rat: parietal cell, smooth muscle, thyroid epithelium, islet cell surface and cytoplasmic antibodies, and β-cell function

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150 age-matched BB/E rats of mixed genotype (DxD, DxND and NDxND matings) were studied as part of a prospective, longitudinal study correlating pancreatic autoimmunity markers, morphology and β-cell function. Groups of 16 animals were sacrificed at 2 week intervals from 30 days of age. Blood samples were taken for measurement of plasma glucose, HbA_1 and insulin, and autoantibody screening and pancreatic autoradiography performed. At 30 and 45 days of age antibodies were detected to parietal cells (PCA) in 6/16 and 8/16 and to smooth muscle (SMA) in 5/16 and 6/16 animals respectively. Islet cell surface antibodies (ICSA) were not found. Between 60 and 105 days the percentage of animals with PCA and SMA was steady at 81% and 31%, respectively, while ICSA were detected with increasing frequency (4/16, 4/16, 9/16 and 8/16 on days 60, 75, 90 and 105, respectively) and these were always associated with PCA or SMA staining. Islet cell cytoplasmic and thyroid epithelium antibodies were not detected in any animal. Metabolic status and β-cell replication were assessed in relation to the autoantibody profile throughout the study. Results suggest that a generalised alteration in humoral immunity precedes and accompanies the appearance of ICSA, previously shown to correlate with subsequent development of insulin-dependent diabetes in the BB/E rat.

52. Impaired insulin removal by the liver after an oral glucose load in simple obesity

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In the present study, insulin and C-peptide levels in peripheral blood in the fasting state and after an oral glucose load were measured in 65 non-diabetic, obese subjects and 65 age- and sex-matched non-diabetic, non-obese controls. In the fasting state both insulin and C-peptide levels were significantly higher in obese subjects than in controls, whereas 1 and 2 h after a glucose load only insulin concentrations were significantly higher in obese subjects. C-peptide to insulin molar ratios, as well as the relationships between incremental areas of the two peptides under the plasma curves, were used as relative measures of the hepatic insulin extraction. In the fasting state, the ratios between C-peptide and insulin were similar in obese and non-obese subjects, whereas they were reduced in the obese subjects after the glucose load. Analogously, the relationships between C-peptide and insulin incremental areas were significantly lower in obese individuals than in controls. However, the comparison of corresponding values of C-peptide and insulin levels and areas after glucose load showed that for the same C-peptide value, insulin values were highest in the obese group. These results suggest that in simple obesity only fasting peripheral hyperinsulinaemia is the result of pancreatic hypersecretion of the hormone, whereas peripheral hyperinsulinaemia occurring after an oral glucose load depends on impaired hepatic insulin removal.

53. Proteinuria determines the prognosis of Type 1 (insulin-dependent) diabetes

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Excess mortality among early onset Type 1 diabetic patients reaches a maximum of 1–2000%. Diabetic nephropathy is the main risk factor in the younger age-groups. We studied the prognostic importance of proteinuria (PU) in relation to excess mortality in 1092 Type 1 diabetic patients (age at diagnosis <31 years, time of diagnosis before 1953 followed until 1981). 372 patients developed PU (328 died), 720 did not develop PU (239 died). Using a Cox-regression model, we studied excess mortality among patients with and without PU, and the prognostic effect of sex, time of diagnosis, age, and calendar-time. In the non-PU-group, excess mortality was constant (150%) and independent of sex, age, and calendar-time. In the PU-group, females had higher excess mortality ($p=0.01$), excess mortality increased until 1968 and then declined ($p=0.005$). Furthermore, excess mortality increased with age to a maximum at age 32 years (6500%). In conclusion, excess mortality is extremely high among patients developing PU, whereas excess mortality is very low in patients without PU. The influence of sex, age, and calendar-time differs in the proteinuric and non-proteinuric-groups.

54. Immunohistochemical study of islet cell carcinomas induced by BK-virus in hamsters

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To characterize the hormonal production of malignant endocrine tumours of the pancreas induced in hamsters by BK-virus, 17 BK-virus-induced islet cell carcinomas were investigated by immunohistochemistry for insulin, glucagon, somatostatin, pancreatic polypeptide (PP) and gastrin. Immunoreactive cells were found in 16 cases, and were more frequent in tumours with trabecular structure. Thirteen cases contained more than one cell type. Insulin cells were present in 16 cases, glucagon cells in 11, somatostatin cells in 7 and PP cells in 6. Insulin cells were the most frequent immunoreactive cell type in 13 tumours and glucagon cells predominated in one case. Insulin cells occupied a central position in the neoplastic clusters, while other cell types were located mostly at the periphery, a distribution reminiscent of that seen in normal islets. Gastrin immunoreactivity was never observed. Moreover, argyrophilic cells revealed by the Grimelius method frequently exceeded the cumulative number of immunoreactive cells, suggesting that additional endocrine cell types occur in these carcinomas. All cell types found in primary tumours could also be detected in liver metastases. In conclusion, we have demonstrated that in this unique model of experimentally induced malignant islet cell tumour all the hormones synthesized in normal pancreatic islets are produced.

55. Evidence of the expression of Class II (HLA-DR) and increased presentation of Class I (HLA-A, B, C) molecules in pancreatic islets in Type 1 (insulin-dependent) diabetes

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Double fluorochrome techniques with monoclonal and polyclonal antibodies have been used to identify infiltrating lymphocytes and individual endocrine cell types in islets, on unfixed 4 μm cryostat sections of the pancreas of a diabetic child who died within 24 h of diagnosis. *Class II antigens*: in control pancreas and areas of the 'diabetic' pancreas devoid of lymphocytic infiltration, islets where β-cells revealed full insulin content, showed minimal staining for HLA-DR. Isolated endothelial-shaped cells at the periphery of islets were positive. Regions infiltrated by activated lymphocytes ('diabetic' pancreas only) showed increased HLA-DR on the capillary endothelium in the periphery and within the connective tissues of individual lobules in affected islets. With the available surface markers it was impossible to identify dendritic cells which may also have been present at these sites. Some β-cells, revealed readily by anti-proinsulin, but negative or weak positive only with anti-insulin, showed HLA-DR in their cytoplasm. *Class I antigens* were expressed in the cytoplasm of all endocrine cells in 'normal' islets. In 'diabetic' islets, a marked increase of Class I antigens correlated with the presence of T cytotoxic/suppressor cells (Leu 2a). We conclude that de novo HLA-DR expression on β-cells and enhancement of Class I antigens are an early event in β-cell activation in Type 1 diabetes.

56. Rat models of diabetic pregnancy for study of fetal lung maturation

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Delay in lung maturation is common in diabetic pregnancy, mainly gestational diabetes. The surge of amniotic fluid surfactant phospholipids (disaturated phosphatidylcholine – DSPC – and phosphatidylglycerol – PG) is delayed, with increased risk of neonatal respiratory distress. The mechanisms were investigated using pregnancy in streptozotocin-diabetic rats. Three groups were obtained: (1) subdiabetic rats – post-absorptive blood glucose only slightly increased (5 mmol/l) but acute glucose tolerance abnormal, hyperinsulinaemia, fetuses slightly enlarged; (2) mildly diabetic – blood glucose 6–11.5 mmol/l, hyperinsulinaemia, fetuses enlarged; (3) severely diabetic – blood glucose >16.5 mmol/l, hypoinsulinaemia, fetuses hypotrophic. At birth DSPC was equally diminished (-20%) in the fetuses of all three groups, in lung tissue as well as in broncho-alveolar fluid. Other phospholipids, including PG and 'membrane' phospholipids, were diminished in the severely diabetic group only. Utilization of glycogen, a presumptive precursor of DSPC, was impaired in the sub- and mildly diabetic groups, but not in the severely diabetic group. In conclusion, (1) surfactant biosynthesis was specifically impaired in fetuses of sub- and mildly-diabetic rats, suggesting a direct involvement of hyperinsulinism; (2) this may be due to abnormal glycogen utilization; (3) decreased PG may be due to hyperglycaemia alone, and (4) sub- and mildly diabetic, but not severely diabetic, pregnant rats are suitable models for studying fetal consequences of maternal diabetes.

57. Prediction of patients' treatment choice using diabetes-specific perceived control and health beliefs measures in a feasibility study of continuous subcutaneous insulin infusion

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In the course of a feasibility study of continuous subcutaneous insulin infusion (CSII), 382 insulin-requiring patients were offered the choice of CSII, intensified conventional treatment (ICT) or conventional treatment (CT). 286 (75%) patients completed newly developed measures of perceived control of diabetes and diabetes-specific health beliefs prior to any change in treatment regimen. The perceived control scales were successful in predicting which patients would choose CSII and which would prefer injection regimens. Patients choosing CSII were more likely to attribute responsibility for outcomes to the treatment recommended ($p<0.001$) and were less likely to feel personal responsibility for outcomes ($p<0.005$), feel in control of outcomes ($p<0.001$) or believe outcomes were foreseeable ($p<0.01$) than were patients choosing injection regimens. The health belief scales distinguished between those patients preferring to continue with the least demanding CT regimen and those who chose intensified regimens (ICT or CSII). Patients choosing CT believed that treatment was more cost effective than did patients wishing to intensify their treatment ($p<0.03$).

58. Chemical stability of insulin: formation of covalent insulin dimers and other higher molecular weight transformation products in intermediate and long-acting insulin preparations

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Chemical transformation of insulin during storage of six different types of intermediate and long-acting preparations was studied by gel chromatography. Covalent insulin dimers were formed in all types of preparations, mainly due to a reaction between an N-terminal amino group in one insulin molecule with a carboxamide group of a glutamine or an asparagine residue in the A-chain of another insulin molecule. In formulations containing protamine, an additional formation of covalent insulin-protamine products occurs. Formation of insulin polymers becomes significant only at storage temperatures higher than 25 °C. These transformations take place to the same extent irrespective of the species of insulin (including human) and the strength of preparation, but vary with the type of formulation. The total transformation in insulin zinc suspension (mixed) during 2 years of storage at 4 °C was 0.25 ± 0.03% (mean ± SEM). The corresponding figures for isophane insulin injection (NPH) were 1.54 ± 0.18%. During storage at 25 °C, the rate of formation was 5–20 times higher than at 4 °C. Compared with insulin, the biological potency of the covalent insulin dimers was 13–15% and, of the insulin-protamine products, 4%. The immunogenicity of the different transformation products in rabbits was not significantly different from MC-insulin.

59. Acetyl-salicylic acid impairs glucose utilization and reduces insulin clearance rate in man

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The effects of acetyl-salicyclic acid (ASA) (3 g/day for 3 days) on glucose utilization (M) and insulin secretion were studied in 13 healthy subjects using hyperglycaemic or euglycaemic insulin clamp techniques. When arterial plasma glucose was acutely raised and maintained >7 mmol/l above fasting level, the early (35 ± 5 mU/l) and late phases (52 ± 7 mU/l) of plasma insulin response were enhanced by ASA (51 ± 9 and 70 ± 7 mU/l, respectively), whereas M was not significantly altered (control 10.9 ± 1.2, ASA 11.7 ± 1.1 $mg \cdot kg^{-1} \cdot min^{-1}$). Plasma C-peptide response (2261 ± 199 pmol/l) was unchanged by ASA (2251 ± 120 pmol/l). Evidence for impaired tissue sensitivity to insulin by ASA was provided by augmented M (15.5 ± 1.6 $mg \cdot kg^{-1} \cdot min^{-1}$), when the early and late insulin responses seen during ASA administration were mimicked by additional intravenous insulin in a hyperglycaemic clamp study. Inhibition of prostaglandin synthesis was not involved in the effects of ASA, since insulin response and M remained within the normal range following treatment with indomethacin, another prostaglandin synthesis inhibitor. In the euglycaemic insulin clamp studies performed with 1 $mU \cdot kg^{-1} \cdot min^{-1}$, insulin concentrations were higher after ASA (103 ± 5 versus 89 ± 4 mU/l). We conclude that in healthy man, ASA (via a prostaglandin-independent mechanism) impairs glucose utilization. This effect is counterbalanced by an augmented plasma insulin response to glucose, which results from an unaffected secretion but reduced clearance rate of insulin.

60. Effect of nifedipine on metabolism of glucose and secretion of insulin in diabetic patients

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The introduction in clinical use of nifedipine, which acts by antagonizing calcium entry into cells, raised the question of impaired insulin secretion and worsening of glucose tolerance. In view of contradictary reports, we re-examined this problem. Three groups of subjects were examined. Group A consisted of five healthy, non-obese subjects. Group B: – eight patients with impaired glucose tolerance and group C: – five diabetic patients treated with oral antidiabetic drugs. All the patients received 80 mg nifedipine/day for 5 days. Before starting treatment and on day 5, an oral glucose tolerance test was performed. For all the patients, there was a medical indication for nifedipine. Blood samples were drawn during the oral glucose tolerance test and were examined for glucose and insulin levels. Glucose and insulin areas before and after nifedipine were compared. Nifedipine did not cause a significant reduction in insulin secretion in the three groups and no worsening of the glucose tolerance in either normal or diabetic subjects. Surprisingly, but similar to results of other workers, nifedipine decreased the glucose area in the group of patients with impaired glucose tolerance: glucose area 1728.94 mmol · min · 1000 before nifedipine decreasing to 1521.26 mmol · min · 1000 ($p < 0.05$). Nifedipine has no detrimental effect on the diabetic state and can be given safely.

61. Smoking does not affect insulin absorption from subcutaneous tissue

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It has been claimed that diabetic patients who smoke need more insulin than non-smoking patients and that the absorption of subcutaneously (SC) injected regular insulin decreases during and after smoking a cigarette. However, the results of the latter study were based on controversial assay methods. Eight non-diabetic habitual smokers were randomly assigned to the following studies: SC injection of (1) 10 U Velosulin without and (2) with smoking, (3) 16 U Mixtard without and (4) with smoking. In the smoking experiments, each subject smoked two cigarettes beginning at time point 0 and at 7.5 min. Insulin was injected at 2.5 min into the thigh. Serum insulin, C-peptide and blood glucose levels, measured at −15, 0, 7.5, 15, 30, 45, 60, 75, 90, 120 and 150 min, were almost identical during the respective experiments with and without smoking throughout the study. In the Velosulin experiments, serum insulin levels increased from mean basal values of 6.0 mU/l (1) and 7.2 mU/l (2) to a maximum of 22.3 (1) and 22.4 mU/l (2) at 45 min. In the Mixtard studies, insulin levels increased from 6.8 (3) and 7.3 mU/l (4) to a maximum of 19.3 (3) and 19.8 mU/l (4) at 120 min. The results of this study show that in habitual smokers smoking has no acute effect on the absorption of subcutaneously injected regular and intermediate acting insulin.

62. Production in the BB rat of a monoclonal antibody specific for the surface of rat insulin-producing cells

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To obtain monoclonal antibodies (MAb) with specificities similar to those of circulating auto-antibodies in the spontaneously diabetic BB rat, 20 fusions were made between lymphocytes of newly diagnosed diabetic BB rats and rat myeloma cell lines (either Y3Ag123 or IR983F). MAb secreted by the resulting clones were characterized by different tests. (1) An ELISA on live rat cells (CELISA); (2) an indirect immunofluorescence on rat cell suspensions (CSIF) and (3) on cryostat rat tissue sections (CrIF). One MAb, IC_2, gave a positive reaction with rat insulinoma cells (RIN5F) but not with other rat cells (thymocytes, hepatocytes and fibroblasts) in the CELISA. (Optical density in the CELISA with RIN5F cells: IC_2: 0.406 ± 0.027; BKG: 0.018 ± 0.009). With CSIF, IC_2 binds only to the surface of a certain proportion of the RIN5F cells and of normal rat endocrine pancreatic cells. No immunofluorescence was observed in rat thymus, liver, brain, kidney, adrenal gland and pancreas with CrIF. Trypsinization of RIN5F cells decreased IC_2 binding suggesting that its specific antigen could be a membrane protein. In conclusion, IC_2 appears therefore to be islet-cell surface specific. Since IC_2 is a IgM and can interact with the complement system, it should be very useful in the determination of the role of anti-islet cell surface antibodies in the pathogenesis of the diabetic syndrome in the BB rat model.

63. Reactive products generated by non-enzymatic glycosylation of collagen covalently trap low density lipoprotein

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Accelerated atherosclerosis with excessive lipid accumulation in arterial extracellular connective tissue matrix characteristically occurs in most diabetic patients, despite circulating lipoprotein levels which are frequently normal. This phenomenon may reflect covalent trapping of LDL molecules by reactive products generated during the process of collagen non-enzymatic glycosylation. We have demonstrated previously that non-enzymatically glycosylated collagen washed free of glucose covalently traps serum albumin and IgG. In the present study, immobilized collagen samples incubated with and without sugar for 14 days were washed free of sugar. Human ^{125}I-LDL was added in varying concentrations to aliquots of glucose-free collagen, and samples were washed with phosphate-buffered saline until no further LDL was eluted. This was followed by exhaustive washing with 4% sodium dodeceryl sulphate. At constant LDL concentrations, covalent trapping increased nearly linearly with the extent of non-enzymatic glycosylation. Trapping by collagen previously glycosylated in G-6-P (50 mmol/l) was 3.5 times greater than control. When varying concentrations of LDL were added LDL binding to phosphate, buffered saline collagen increased as a function of LDL concentrations. Over the same range of LDL concentration, covalent trapping of LDL by previously glycosylated collagen averaged 3.2 times as much as control. In vivo, this mechanism may contribute to the accelerated atherosclerosis of diabetes.

64. Evaluation of insulin resistance in human cirrhosis before and after porto-systemic surgical anastomosis

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To evaluate the influence of porto-systemic surgical shunting on insulin resistance, we examined 18 male impaired-glucose-tolerant cirrhotic patients (liver biopsy), within 110% ideal body weight, aged under 50 years, with portal hypertension and a combined receptorial and post-receptor defect studied in vivo (euglycaemic clamp) and in vitro (insulin binding and glucose transport). The following tests were performed before and 1 month after surgery: euglycaemic hyperinsulinaemic glucose clamp infusing human insulin (Humulin, Lilly) at ~100, ~1,000 and ~10,000 mU/l insulin levels (six subjects at each level); ^{3}H-glucose infusion (0.2 μCi/min for 240 min) started 120 min before clamp to evaluate glucose production; study of insulin binding on circulating monocytes. No significant variation (paired t-test) between values (mean ± SEM) before and after surgical shunting were recorded: fasting plasma glucose (mg/dl) 89.0 ± 3.7 versus 88.5 ± 7.6; fasting plasma insulin 31.1 ± 4.5 versus 20.7 ± 4.7 mU/l; fasting plasma C-peptide 2.6 ± 0.2 versus 2.8 ± 0.4 ng/ml; M index ($mg \cdot kg^{-1} \cdot min^{-1}$) 2.52 ± 0.32 versus 2.37 ± 0.33 at ~100 mU/l; 7.67 ± 0.64 versus 7.48 ± 0.71 at ~1,000 mU/l; 11.69 ± 0.70 versus 12.5 ± 0.62 at ~10,000 mU/l; basal glucose production ($mg \cdot kg^{-1} \cdot min^{-1}$) 2.19 ± 0.31 versus 2.75 ± 0.62; residual glucose production at ~100 mU/l, 0.39 ± 0.27 versus 1.06 ± 0.62; specific insulin binding 2.45 ± 0.32 versus 2.87 ± 0.22%. These results suggest that (1) portal-systemic shunt does not modify the mechanism of insulin resistance in cirrhosis; (2) hepatic cellular damage seems to play a major role in insulin resistance by hyperinsulinism.

65. Uptake of glucose and insulin sensitivity of rat brown adipose tissue

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The ability of brown adipose tissue (BAT) to utilize glucose as substrate, and its insulin sensitivity in vivo were investigated using the euglycaemic hyperinsulinaemic clamp. Different physiological conditions (19 diabetic-pregnant, 12 diabetic-lactating and fed or 48 h fasted virgin rats), characterized by various basal insulin levels (respectively, 102 ± 7, 50 ± 5, 108 ± 16 and 30 ± 5 mU/l), and various insulin sensitivities were studied. During pregnancy and lactation, glucose is preferentially directed to the conceptus or the mammary gland, and other peripheral tissues become insulin resistant. In the basal state, the uptake of glucose by BAT, measured with a ^{3}H-2 deoxyglucose (2DG) technique (adaptated from Sokoloff, 1977), was not modified by pregnancy or lactation (22 ± 3 μmol glucose/mg BAT per min), but was increased 12-fold after fasting (205 ± 39 μmol glucose/mg BAT per min). During the euglycaemic clamp, at maximal insulin concentration (4000 mU/l), the uptake of 2DG by BAT was increased 40-fold in virgin rats, but was increased 8-fold in pregnant rats, and 18-fold in lactating rat. In conclusions (1) glucose is a substrate for BAT: (2) BAT is extremely sensitive to insulin in vivo and (3) BAT insulin responsiveness changes according to physiological conditions.

66. Virus-induced diabetes in mice: early deposits of immunoglobulin in the islets and changes in the percentage of cytotoxic/suppressor cells

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It has been found previously that the encephalomyocarditis (EMC) virus-induced diabetes in BALB/c/BOM mice is thymus-dependent, although the precise mechanism is unknown. Since the lymphocytic infiltration in the islets is modest, we have looked for possible indications of humoral immune mechanisms. Using fluorescence microscopy, the presence of immunoglobulins in the islets could be shown 3 days after EMC-virus inoculation, gradually disappearing by about day 14. The antibody deposit is scattered throughout the islets and their precise target is unknown. Virus antibodies in peripheral blood could not be detected until day 5 after virus inoculation, whereas virus could be isolated from day 3. Beginning from day 5 about one-third of the mice developed severe hyperglycaemia with blood glucose levels up to 35 mmol/l. Lymphocyte subsets of spleen cells were measured using a fluorescence activated cell sorter. Six days after virus inoculation the mean percentage of Lyt 2-positive (cytotoxic/suppressor) cells decreased below the value for control mice ($p < 0.05$), but increased significantly ($p < 0.02$) 2 weeks later.

67. Influence of a diabetes education programme on the degree of knowledge, behaviour and glycaemic control of Type 1 (insulin-dependent) diabetes

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The aim of this study was to evaluate the efficiency of a diabetes education programme (5 days in hospital) by comparing the evolution of knowledge, behaviour and the level of HbA_{1c} in 85 Type 1 diabetic patients before and 6 months after the programme (without major modification of insulin therapy). The indices of knowledge and behaviour (defined as the percentage of correct answers to a written questionnaire) respectively improved from 63.9 ± 2.5% and 59.7 ± 2.9% before the education programme to 83.3 ± 1.6% and 82.9 ± 1.2% 6 months later (mean ± SEM, $p < 0.001$). Concomitantly, the level of HbA_{1c} significantly decreased from 12.5 ± 0.3% before to 10.9 ± 0.3% 6 months later ($p < 0.001$). A significantly lower value of HbA_{1c} was observed 6 months after the education programme in patients with a residual insulin secretion (C-peptide ⩾ 0.05 pmol/ml) than in totally insulin-dependent subjects ($p < 0.01$). However, no correlation was found, in a given patient, between the indices of knowledge, behaviour and glycaemic control after 6 months. In conclusion, our results indicate that a diabetes education programme can improve both the knowledge and behaviour indices as well as the HbA_{1c} levels in Type 1 diabetic patients. However, the evaluation by questionnaires cannot predict the quality of control, indicating the requirement for continuous care.

68. Excercise-induced abnormalities of left ventricular function in Type 1 (insulin-dependent) diabetic patients with recent onset of disease

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The aim of the present study was to evaluate left ventricular function in 60 Type I diabetic patients, aged 14–34 years, with known duration of diabetes from 8 months–4 years. None of the patients was ketoacidotic and all were in moderate metabolic control ($HbA_1 < 10\%$). The patients were separated into three age groups (< 20, 20–30, > 30 years; n = 20 for each group) and were studied by M-mode echocardiography. Under basal conditions, the diabetic patients had increased ($p < 0.05$) systolic and diastolic dimensions of left ventricle, and reduced systolic septal excursions ($p < 0.001$), compared with age-matched control groups (n = 25 each) suggesting reduced contractility. The abnormalities increased with age, so that they were more pronounced in older subjects. Major echo abnormalities were detected after multi-stage bicycle ergometric exercise, which revealed reduced increases ($p < 0.01$) in both systolic septum excursions and diastolic diameters. Heart rate increased significantly less ($p < 0.01$) after exercise in diabetic compared with control subjects. These data indicate that diabetic patients without cardiac lesions have major echocardiographic abnormalities that increase with age and exercise. The presence of preclinical cardiomyopathy in diabetic patients of recent onset is also suggested by the presence of septal pseudohypertrophy (increased dimension but decreased contractility).

69. Glucocorticoids and insulin resistance: comparison between the in vivo effects of betamethasone and dexamethasone

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To evaluate whether a relationship exists between glucocorticoid molecular structure and insulin resistance, ten normal subjects were divided into two equal groups: one was given betamethasone and the other dexamethasone, which differ in that the C_{16}-CH_3 is in β and α positions, respectively, at the same dose (3 mg/day for 3 days). Plasma glucose (PG), plasma insulin (IRI), insulin binding to monocytes were determined before and after steroid administration. Betamethasone and dexamethasone induced a comparable insulin resistance both in basal levels (PG: 4.7 ± 0.05 versus 5.38 ± 0.05 mmol/l, $p < 0.05$; 4.5 ± 0.01 versus 5.38 ± 0.1 mmol/l, $p < 0.025$; IRI: 13 ± 1 versus 38 ± 8 mU/l, $p < 0.05$; 12 ± 1 versus 22 ± 2 mU/l, $p < 0.05$) and after intravenous glucose tolerance test (mean PG area: 0.27 ± 0.03 versus 0.19 ± 0.02 $mmol \cdot l^{-1} \cdot h^{-1}$, $p < 0.01$; 0.13 ± 0.03 versus 0.18 ± 0.02 $mmol \cdot l^{-1} \cdot h^{-1}$, $p < 0.025$; mean IRI area: 3.5 ± 0.2 versus 6.8 ± 0.4 $mU \cdot l^{-1} \cdot h^{-1}$, $p < 0.025$; 2.3 ± 0.2 versus 5.1 ± 0.3 $mU \cdot l^{-1} \cdot h^{-1}$, $p < 0.005$) but induced opposite effects on insulin receptors (7 ± 1 versus 9.4 ± 0.6%, $p < 0.01$; 7.2 ± 0.4% versus 5.6 ± 0.1%, $p < 0.05$) before and after treatment. Scatchard analysis and average affinity profile

showed that number but not insulin receptor affinity varied. In conclusion, since betamethasone and dexamethasone have comparable insulin resistance but opposite effects on insulin receptors, insulin resistance may be a post-binding event; perhaps small variations in the steroid molecular structure affect interaction between insulin and receptors. This suggests that steroids directly affect synthesis and insulin receptor turnover.

70. Nocturnal spikes in growth hormone secretion cause the dawn phenomenon

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To determine the mechanism for the dawn phenomenon, an early morning increase in plasma glucose concentrations and insulin requirements commonly seen in both Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes, six (C-peptide deficient) Type 1 diabetic subjects were managed solely by regular insulin for 30 h, rendered euglycaemic by closed-loop intravenous insulin administration (Biostator) and were then infused intravenously with insulin at a constant rate ($0.15\ mU \cdot kg^{-1} \cdot min^{-1}$) from 2400 h until 0800 h on four separate occasions. In control experiments, plasma glucose remained stable until 0300 h (5.5 ± 0.5 mmol/l) and then rose progressively to 12.1 ± 1.7 mmol/l at 0800 h ($p < 0.001$) due to an increase from 10 ± 2 to $16 \pm 3\ mmol \cdot kg^{-1} \cdot min^{-1}$ ($p < 0.01$) in glucose production (3-^{3}H glucose) which began shortly after 0200 h. These changes in plasma glucose and glucose production were unaffected by combined α-β adrenergic blockade (phentolamine + propranolol) but were completely prevented by selective growth hormone suppression (somatostatin + glucagon replacement). Simulation of nocturnal plasma growth hormone patterns observed in control studies by hourly intravenous growth hormone injections (10–100 μg) during infusion of somatostatin + glucagon completely reproduced the hyperglycaemia and increased glucose production observed in control studies. We conclude that increases in glucose production due to nocturnal spikes in growth hormone secretion are responsible for the dawn phenomenon.

71. Subcellular distribution of protein carboxymethylase and its substrates in rat pancreatic islets

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Protein carboxymethylation may play a rôle in stimulus-secretion coupling in islets. This work aimed at investigating protein carboxymethylase (PCM) activity, methyl proteins (MAP) content and electrophoretic properties of carboxymethylated proteins, in subcellular fractions of isolated rat islets (succinate cytochrome c-reductase and insulin were used as markers). Specific PCM activity (nmol ^{3}H-methyl/μg protein), determined by incubating fractions in the presence of ^{3}H-SAM and gelatine, was higher in microsomal-cytosolic (4,19) and nuclear (6,89) than in granular (1,19) and mitochondrial (1,78) fractions. Specific MAP capacity (nmol ^{3}H-methyl/g protein) determined as for PCM activity, but using PCM purified from bovine adrenal medulla instead of gelatine, was higher in granular (1,91) and microsomal-cytosolic (5,50) than in nuclear (0,045) and mitochondrial (0,35) fractions. Electrophoresis (polyacrylamide gel urea-acetic acid system) of the fractions (treated as for MAP capacity determination) showed several peaks of radioactivity (^{3}H methanol/2 mm gel slice) more evident in granular fractions. These carboxymethylated proteins showed molecular weights from 12,000 to 40,000. In conclusion: (1) rat pancreatic islets contain PCM activity and MAP capacity; (2) subcellular distribution of PCM and MAP is similar to that described for pituitary and adrenal medulla; (3) MAPs represent a heterogenous group of low-molecular-weight proteins.

72. Conformational changes of urinary albumin as detected by isoelectric focussing in high denaturating media: studies in diabetic functional nephropathy

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Urinary albumin (U. alb) includes two main bands with different pI's, the more anionic of which (pI = 4.1) is thought to be the more glycosylated. Comparison of motility of U. alb at isoelectric focussing in non-denaturating media and in high molar concentration of urea (8 mol/l) provides a direct evaluation of the conformational status of U. alb since urea induces a complete unfolding of the molecule. This model was applyied to U. alb from five diabetic patients with functional nephropathy, after the protein had been purified with pseudo-ligand chromatography on Affi-Gel Blue. Isoelectric focussing was performed in ultra-thin slabs containing glycerol or urea 8 mol/l and proteins stained with photochemical silver. While U. alb with a pI of 4.7 showed a shift of 2 unit of pH when exposed to urea, the more anionic band produced only a faint increase of its pI generating numerous microheterogenous bands between 4.1 and 4.6. The same pattern was generated by urinary glycosyl-albumin as purified by Con-A Sepharose. These observations suggest that the more anionic U. alb is deranged in its three-dimensional conformation while becoming more glycosylated. They also explain the influence of glycosylation on the renal handling of albumin.

73. Influence of hyperglycaemia per se on forearm glucose metabolism in Type 2 (non-insulin-dependent) diabetes

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A remarkable forearm glucose uptake has been demonstrated in normal man even during acute insulin deficiency. To quantify non-insulin-stimulated glucose uptake in Type 2 diabetes, six normal-weight Type 2 diabetic patients (mean ± SEM age 48 ± 2 years) and six normal subjects (age 40 ± 2 years) were studied. Diabetic patients discontinued oral hypoglycaemic treatment for 2 weeks. The day before the study, normoglycaemia was achieved by continuous subcutaneous insulin infusion, which was discontinued 4 h before the experiment. Somatostatin (0.7 mg/h) and a variable amount of glucose were infused into an antecubital vein. Simultaneous blood samples from the controlateral brachial artery and a deep forearm vein were withdrawn in the basal state and at 30-min intervals for 90 min. Arterial blood glucose rose to 10.2 ± 0.28 and 11.2 ± 0.33 mmol/l in control and diabetic subjects respectively. Forearm glucose uptake (arterial-venous difference × blood flow) rose from 83 ± 10 in the basal state to $478 \pm 100\ \mu g \cdot 100\ ml^{-1} \cdot min^{-1}$, at 90 min in controls and from 174 ± 22 to $730 \pm 134\ \mu g \cdot 100\ ml^{-1} \cdot min^{-1}$ in diabetics. Plasma insulin levels were similar in the two groups (< 5 U/l) during somatostatin infusion. In conclusion, non-insulin-stimulated glucose uptake by forearm tissues is not impaired in Type 2 diabetic patients and therefore it has no rôle in the defective peripheral glucose utilization.

74. Presence of a Zn^{2+}-activated ATP-pyrophosphohydrolase in mouse pancreatic islets

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It has been shown that pancreatic islets possess an extragranular Zn^{2+}-pool of considerable size (1–4 mmol/l), which can be regulated independently of insulin secretion. However, no suggestions have been made about the metabolic function of extragranular Zn^{2+} in pancreatic islets. We report the presence of a Zn^{2+}-stimulated ATP-pyrophosphohydrolase which liberates $^{32}PP_i$ from γ-^{32}P-ATP. The enzyme could be demonstrated both in a 27,000 *g* particular fraction and the supernatant. In the absence of metal-ions the activity in the supernatant fraction was 0.32 ± 0.09 pmol/min per μg protein (ATP concentration: 5 μmol/l). In the presence of EGTA (1 mmol/l), $CaCl_2$ (2 mmol/l) and $ZnCl_2$ (2 mmol/l) increased the activity 7–10-fold in an additive manner. Mg^{2+}, Mn^{2+}, Ba^{2+}, Sr^{2+}, Ni^{2+}, Na^+ and K^+ had no effect on the basal activity, but in the presence of $ZnCl_2$ (2 mmol/l), 1 mmol/l concentrations of Ni^{2+}, Mn^{2+}, Ba^{2+} and Sr^{2+} potentiated the activity 184, 145, 58 and 40% respectively, whereas Mg^{2+}, Na^+ and K^+ had no effects. The enzyme activity was not dependent on calmodulin. Cyclic AMP (1 mmol/l) and 3-isobutyl-1-methyl-xanthine (1 mmol/l) potentiated the Ca^{2+}, Zn^{2+}-activated enzyme by about 30%. This Zn^{2+}-activated pyrophosphohydrolase may play a role in the metabolism of nucleotides and oligonucleotides in islets.

75. Insulin resistance in the second half of pregnancy: correlation with insulin receptors and gestational hormones

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Pregnant women frequently show high glucose and insulin values after an oral glucose tolerance test (GTT) and insulin resistance (IR). The importance of gestational hormones and insulin receptors in IR is still unexplained and existing data are discordant. We have studied glucose tolerance, insulin receptors, IR and plasma concentrations of

cortisol, human placental lactogen (HPL), oestradiol and progesterone in 12 pregnant women and in 12 control subjects. Glucose tolerance was evaluated by an oral GTT. Insulin receptors were studied on blood monocytes. IR was evaluated infusing glucose (23.3 mmol/min), insulin ($0.77\,mU \cdot kg^{-1} \cdot min^{-1}$) and somatostatin (500 µg/h). Since similar steady state plasma insulin was maintained in all subjects, steady state plasma glucose concentration (SSPG) was considered an index of IR. Pregnant women during the oral GTT presented higher ∫ blood glucose (30.8 ± 4.7 versus 28.3 ± 3.5 mmol/l– $p < 0.05$) and ∫ insulin values (401 ± 156 versus 228 ± 131 mU/l; $p < 0.005$) compared with control subjects. Insulin bound was reduced (1.73 ± 0.16 versus 1.99 ± 0.19; $p < 0.05$) because of a reduction of receptor number with normal affinity, and correlated with basal insulin concentration ($r = -0.87$; $p < 0.05$). SSPG was increased (6.6 ± 1.6 versus 4.2 ± 1.1 mmol/l; $p < 0.001$) but not correlated with insulin bound, cortisol, HPL and progesterone. A correlation was found between SSPG and oestradiol ($r = 0.92$; $p < 0.01$). IR in pregnancy is not dependent on reduced insulin bound, but it is the consequence of non-receptorial factors which are correlated with oestradiol concentration.

76. Is there a mechanism regulating cell surface insulin receptors in experimental hypoinsulinaemic states and insulin-dependent diabetes?

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In hyperinsulinaemic states, cell surface insulin receptors are frequently down-regulated; our previous work suggested that this process is regulated by relative rates of receptor endocytosis and recycling. In the present study we have asked whether similar control mechanisms are operative in hypoinsulinaemic states. Since insulin-dependent diabetes is accompanied by an increased number of surface insulin receptors, we looked for an alteration of ^{125}I-insulin internalization in hepatocytes from streptozotocin-diabetic rats. Cells from six control, six streptozotocin-diabetic and six insulin-treated streptozotocin-diabetic rats were incubated for 2 h at 37 °C with ^{125}I-insulin and the percentage of radioactivity inernalized was determined by quantitative electron microscope autoradiography. At 30, 60, 90 and 120 min, the percentage of autoradiographic grains localized inside hepatocytes of diabetic animals was significantly lower than that found inside hepatocytes of the two other groups. Preliminary studies suggest that this internalization defect is ligand-specific since hepatocytes of diabetic rats internalize ^{125}I-glucagon at a higher rate than hepatocytes of normal animals. Further, we have found the same insulin internalization defect in freshly isolated monocytes from two untreated insulin-dependent diabetic patients. These data suggest a mechanism whereby target cells regulate their cell surface insulin receptor to a diminished hormonal stimulus.

77. Pyruvate dehydrogenase activity in hearts from obese mice: effect of fatty acid oxidation and weight

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The activity of the pyruvate dehydrogenase complex (PDHC) is decreased in hearts from mice with chronic gold thioglucose-induced obesity. In examining the development of this change, at one week PDHC was increased in hearts from injected mice (1.20 ± 0.12 ($n = 8$) versus 0.75 ± 0.13 ($n = 7$) U/g wet weight in controls). By 3 weeks post-injection, the corresponding values for PDHC were 0.90 ± 0.11 ($n = 10$) and 0.80 ± 0.17 ($n = 7$) U/g wet weight. At 4 weeks post-injection, PDHC in injected mice fell to 29% of control values (0.29 ± 0.06 U/g wet weight) coincident with maximum weight gain. The decrease in PDHC was maintained in subsequent weeks of obesity. Fasting serum insulin was not elevated at 4 weeks in these mice, but by 8 weeks it was 211 ± 36 ($n = 16$) versus 34 ± 3 ($n = 5$) mU/l in control mice. Insulin receptor numbers decreased progressively to 50% of control values. A single oral dose of 2-tetradecylglycidic acid (M_cN3802, an inhibitor of β oxidation) returned PDHC in heart muscle from obese mice to the level found in control mice. These studies show that the decrease in PDHC in obese mice may be due to increased fatty acid oxidation.

78. Abnormal colour vision and reliability of blood glucose self-monitoring

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Abnormal colour vision is a common feature in diabetes. The aim of this study was to investigate its influence on the reliability of blood glucose monitoring without a meter. We have investigated 103 insulin-treated patients (mean age 44 ± 14 years; male 61%) and 49 control subjects (mean age 39 ± 14 years; males 39%). Colour vision status was evaluated by an automated Farnsworth-Munsell 100-hue test quantifying abnormalities and exhibiting axial defects on a graph. Blood glucose monitoring accuracy was assessed by reading 30 precalibrated 20–800 BM test strips checked against manufacturer's colour scale. Reading errors were of three types: (A) within right colour interval, (B) in adjacent block interval, (C) beyond. Diabetics made twice as many errors as controls: 3.8 ± 2.2 versus 2.1 ± 1.8 and 4.2 ± 2.4 versus 2.2 ± 2.4 (mean ± SD) for types A and B, respectively. Only diabetic patients made type C errors. Patients with axial defect (21%) made twice as many type B errors as patients without (5.2 ± 1.4 versus 3.9 ± 2.6; $p < 0.02$). Reading accuracy of patients without axial defect was strongly correlated to 100-hue numerical score ($r = 0.52$ for type A errors; $r = 0.592$ for type B errors; $p < 0.001$) with patients having dyschromatopsia consistently hesitant about their readings. Our results suggest that dyschromatopsia should be ruled out by colour vision testing before recommending blood glucose monitoring without a meter.

79. Influence of the metabolic control on systolic time intervals in type 1 (insulin-dependent) diabetes mellitus after dynamic exercise

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In subclinical diabetic cardiomyopathy previous reports did not conclusively correlate the altered cardiac performance with metabolic parameters. Fifteen Type 1 diabetic normotensive subjects, aged 16–32 years, without any clinical or instrumental evidence of heart disease, were studied. Signs of diabetic microangiopathy were absent. Systolic time intervals (STI) and metabolic parameters (blood glucose; non-esterified-fatty acid (NEFA), lactate) were evaluated at rest and after dynamic exercise during poor control mean ± SEM: mean amplitude glucose excursion (MAGE, 8.02 ± 0.72 mmol/l), and during good control obtained with continuous subcutaneous insulin infusion (MAGE 3.46 ± 0.68 mmol/l). Resting values of STI were normal during both poor and good metabolic control. After exercise pre-ejection period/left ventricular ejection time ratio (PEP/LVET) increased only during poor metabolic control (0.369 ± 0.01 versus 0.340 ± 0.008 at rest; $p < 0.001$) as result of an increased PEP; conversely, good metabolic control induced a decrease in PEP/LVET ratio in response to exercise (0.321 ± 0.005 versus 0.347 ± 0.009 at rest; $p < 0.001$). The exercise-induced NEFA utilization did not occur during poor metabolic control but occurred during good control (0.082 ± 0.013 versus 0.51 ± 0.14 mmol/l, at rest; $p < 0.005$). In conclusion, testing of STI during exercise might be useful in the detection of preclinical diabetic cardiomyopathy. The decrease of cardiac functional reserve, observed during poor metabolic control, might be related to an altered energetic fuel utilization.

80. Platelet fibrinogen binding in diabetes mellitus

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Studies were carried out to determine whether the hyperaggregable platelets from diabetic patients bind more fibrinogen than normal platelets when prostaglandin/thromboxane formation is suppressed (aspirin). We found the following: pre-treatment with aspirin reduced collagen- or thrombin-induced binding to platelets from non-retinopathy diabetic patients to the values seen in control subjects. At variance with this, aspirin could not normalize binding in platelets obtained from diabetic patients with retinopathy. The combination of aspirin + apyrase (an ADP scavenger) almost completely inhibited binding and aggregation of platelets from normal or non-retinopathy subjects exposed to collagen or thrombin, whereas it could only partially affect binding and aggregation of platelets from patients with retinopathy. By using a monoclonal antibody (B59.2) to the platelet receptor for fibrinogen, we determined that this receptor was quantitatively and qualitatively the same on platelets from normal and diabetic subjects. We conclude that increased fibrinogen binding and hyperaggregability of platelets from diabetic patients without retinopathy is related to their capacity to form more prostaglandin and endoperoxide/thromboxane than normal platelets. In contrast, hyperaggregability and increased binding of platelets from patients with retinopathy appears at least in part to be related to a mechanism independent of ADP secretion and thromboxane synthesis.

81. Moderate dietary intake of sucrose does not affect metabolic control in near-normoglycaemic pump-treated Type 1 (insulin-dependent) diabetic patients

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The effect of dietary sucrose intake on metabolic control was studied in eight Type 1 diabetic patients (seven females, aged 25–43 years, duration of diabetes 6–18 years, basal C-peptide levels below detection limit, normal body weight, and normal serum lipids) treated by continuous subcutaneous insulin infusion for more than 1 year. After a 4-week run-in period, the patients were asked to use sodium-cyclamate or sucrose as sweeteners for 1 month each in randomized order. The intake of sucrose (as assessed from the patients recordings) was 25 g/day. The intake of cyclamate (assessed by counting the cyclamate-tablets and liquids that had been drunk) was 0.35 g/day. Biweekly, post-prandial blood glucose, serum lipids, body weight, and HbA_{1c} levels (normal range 4–7.8%) were determined. Results (mean): body weight: 65 kg during the run-in period, 65 kg during the sucrose-period (SP), 65.4 kg during the cyclamate-period (CP); blood glucose: 6.9 mmol/l, 5.8 mmol/l (SP), and 6.9 mmol/l (CP); HbA_{1c}: 7.88%, 7.59% (SP), and 7.62% (CP); serum cholesterol: 5.07 mmol/l, 5.16 mmol/l (SP), and 5.2 mmol/l (CP); triglycerides: 1.01 mmol/l 0.88 mmol/l (SP), and 0.9 mmol/l (CP). In conclusion, moderate dietary intake of sucrose (in accordance with the proposals of the British National Advisory Committee on Nutrition Education for the general population released in 1983) did not impair diabetic control in near-normoglycaemic, normolipaemic, normal-weight, pump-treated Type 1 diabetic patients.

82. Effect of serum lymphocytotoxic activity on immunoregulatory T lymphocytes in Type 1 (insulin-dependent) diabetes

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Lymphocytotoxic activity (LCA) was tested in sera from 13 patients with Type I diabetes of recent onset and 33 of their healthy family members. The aims of the study were: (a) to define the frequency and cell specificity of this potentially immunoregulatory phenomenon and (b) to identify whether consanguineous and non-consanguineous contacts exhibited similar lymphocytotoxicity. Sera were screened against peripheral mononuclear cells then further tested against T lymphocytes and purified helper/inducer (OKT4) and cytotoxic/suppressor (OKT8) cells. Age-matched control groups included parents of children undergoing urgent hospitalisation for non-immunological conditions. Significant LCA was found in 77% of diabetic patients (mean: 25% cell killing; range 1–69%); 79% of consanguineous relatives (mean: 22%) and each of four non-consanguineous contacts (21%). No significant cytotoxicity was observed with control sera (i.e. cell killing $< 5\%$). Lymphocytotoxicity was observed predominantly against OKT4 cells, although some degree of killing was also detected against OKT8 cells. The finding of LCA in both non-consanguineous and consanguineous contacts suggests a viral basis for the reaction, as LCA is known to occur with viral infection. LCA against T cell subsets could influence immunoregulation in Type 1 diabetes.

83. Cyclic AMP-dependent protein phosphorylation in islets of Langerhans

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We have used two approaches to investigate the substrates for cyclic AMP-dependent protein kinase (cyclic AMP-PrK) in rat islets. Subcellular fractions of islet homogenates were incubated with [γ-^{32}P]ATP and the catalytic subunit of cyclic AMP-PrK; phosphopeptides were analysed by SDS-gel electrophoresis and autoradiography. The molecular weights of the major endogenous protein substrates in the various fractions were: 25,000, 30,000, 57,000, and 60,000 (cytosolic); 17,000, 23,000 and 53,000 (24,000 *g* pellet); 17,000, 32,000 and 45,000 (190,000 *g* pellet). To determine which of these proteins may be substrates for cyclic AMP-PrK in intact islets, batches of islets were incubated with $^{32}P_i$ for 1 h in the presence of glucose 10 mmol/l and then islet cyclic AMP was elevated by forskolin, a stimulator of adenyl cyclase. Within 5 min, forskolin caused increased labelling of the 30,000 Dal cytosolic, and the 23,000 and 32,000 Dal particulate peptides; a rapid decrease in phosphorylation of an 18,000 Dal cytosolic peptide was also found. In addition, rather slower phosphorylation of histone H3 was observed. These studies demonstrate that the potentiation of insulin secretion that occurs when islet cyclic AMP is elevated is accompanied by phosphorylation of specific islet substrates for cyclic AMP-PrK; the data are consistent with the hypothesis that protein phosphorylation may play a role in the control of insulin secretion.

84. Abnormal neural metabolism of substance P in diabetic neuropathy

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Since substance P release from primary sensory neurons mediates the cutaneous flare that follows application of noxious stimuli to the skin, we studied the substance P content of human diabetic and non-diabetic nerves as well as the flare response to intradermal injection of substance P agonists in control subjects, non-neuropathic diabetic patients and diabetic patients with severe neuropathy. Homogenates of nerves from amputated legs of diabetic patients contained less radioimmunoassayable substance P (0.32 ± 0.12 fmol/mg) than did nerves from non-diabetic patients (1.27 ± 0.30 fmol/mg, $p < 0.01$). The forearm cutaneous flare following histamine injection (an indirect stimulus to substance P release) was profoundly decreased in neuropathic diabetic patients (11.4 ± 1.8 cm^2, $n = 33$) when compared with normal subjects (48.0 ± 11.7 cm^2, $n = 11$, $p < 0.005$) or non-neuropathic diabetics (43.1 ± 5.4 cm^2). Similarly, the flare in response to capsaicin injection (a direct releaser of substance P) or to injection of synthetic substance P also was markedly decreased in the neuropathic diabetics compared with the other groups. We conclude that there is a diminished neural content of substance P and decreased cutaneous vascular response to this peptide in diabetic neuropathy, and that these abnormalities may predispose such patients unknowingly to injure their skin.

85. Impaired insulin sensitivity of peripheral and hepatic glucose metabolism after nocturnal hypoglycaemia in Type 1 (insulin-dependent) diabetes

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To determine the contribution of insulin resistance to rebound hyperglycaemia, C-peptide deficient diabetic patients were studied twice, once after induction of hypoglycaemia (2.4 ± 0.2 mmol/l) at 01.00 h by intravenous insulin infusion ($1.5\ mU \cdot kg^{-1} \cdot min^{-1}$ for, 110 min), once without hypoglycaemia (controls; plasma glucose 8.3 ± 0.4 mmol/l). Before and after hypoglycaemia, plasma glucose levels were maintained constant at 7–9 mmol/l by intravenous insulin at variable rates. Six hours after hypoglycaemia, insulin sensitivity was assessed using the glucose clamp technique (insulin infusion 20 mU/m^2 per min for 2 h, and glucose 20% to maintain basal glucose levels), combined with 3-^{3}H-glucose infusion. Plasma free insulin levels were similar in both studies before and 6 h after hypoglycaemia (21 ± 7 versus 16 ± 2 mU/l). During the clamp they were 36 mU/l in both studies. Plasma adrenaline, cortisol and growth hormone increased significantly within 30 min after hypoglycaemia and declined thereafter. Six hours after hypoglycaemia, basal glucose production and clearance were not significantly different compared with the control study. However, during the clamp glucose production was less suppressed (65% versus 89%, $p < 0.03$) and glucose clearance was less enhanced by insulin (by 6% versus 19%, $p < 0.02$) after hypoglycaemia. Thus, impaired insulin sensitivity of peripheral and hepatic glucose metabolism, rather than disturbed basal glucose kinetics, contribute to the derangement of glucose homeostasis after nocturnal hypoglycaemia.

86. The effect of hypoglycaemia upon visual function

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Disturbances of vision commonly accompany hypoglycaemia. To investigate possible mechanisms we measured visual acuity and refraction, colour vision (100-hue test), visual evoked responses (VER), EEG frequency analysis and psychometry (digit recall) during controlled hypoglycaemia produced by an intravenous insulin infusion. Six male volunteers (aged 24–47 years, mean 33 years) and five Type 1 (insulin-dependent) subjects (aged 19–38 years, mean 32 years) were studied. Measurements were taken at baseline, during descent into hypoglycaemia, at the glucose nadir ($= 1.5$ mmol/l) and upon recovery. During hypoglycaemia refractive changes occurred in 7 out of 22 eyes but corrected acuity was unchanged. Colour vision was significantly impaired ($p < 0.01$). Baseline VER latencies were normal in both groups but lengthened significantly with hypoglycaemia (mean increment 11 ms, $p < 0.001$) becoming abnormal in 8 out of 11 subjects. Quantitative EEG analysis demonstrated slowing with a power

density spectral shift from fast-α to slow-α ($p < 0.002$) and δ which correlated with VER latency ($r = 0.69$, $p < 0.002$) and amplitude ($r = 0.71$, $p < 0.002$) changes. Psychometric performance was unchanged. These diverse findings have practical implications. Colour vision changes may impair ability to read reagent strips by eye. VER measurements in diabetic patients may be misleading if hypoglycaemia is present. EEG changes are a sensitive index of cortical dysfunction and provide a theoretical basis for developing a portable device to detect early hypoglycaemia.

87. Assessment of activity of subcutaneously injected insulin using the glucose clamp technique

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The glucose clamp was used to study the activity of Actrapid insulin (100 U/ml) injected subcutaneously into the abdominal wall to examine the influence of (1) pre-mixing with Monotard insulin and (2) injecting as a bolus dose compared with multiple small doses. Each study was conducted over 5 h. Twelve non-obese insulin-treated diabetic patients were studied (mean age 42 years). Eight subjects were given Actrapid (10 U) and Monotard (20 U) separately and after premixing in a syringe and left standing for 5 min. The total glucose (mean ± SEM) administered was 87.2 ± 9.7 g separate, compared with 63.7 ± 7.3 g premixed ($p < 0.05$). Fasting plasma glucose was 7.5 ± 1.7 and 7.8 ± 2.8 mmol/l, respectively. Seven subjects were studied following injection of Actrapid (0.2 U/kg) injected either as a bolus dose or divided into four small doses. The total glucose (mean ± SEM) administration was 88.3 ± 10.8 g for bolus study and 110.8 ± 11.5 g for multiple dose study ($p < 0.05$). Fasting plasma glucose was 6.5 ± 2.2 and 6.4 ± 2.3 mmol/l, respectively. These differences were not explained by differences in serum insulin concentration. These results demonstrate a diminished effect of subcutaneously injected Actrapid when pre-mixed with Monotard and an increased effect when given as multiple small doses compared with a bolus dose.

88. A novel glucagon-like peptide from guinea pig pancreas

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The aim of the study was the characterisation of the glucagon-like peptides in guinea pig pancreas. Analysis of extracts of guinea pig pancreas by gel filtration and ion-exchange chromatography indicated that the material with glucagon-like immunoreactivity (GLI) was larger and more basic than glucagon and showed approximately a 10-fold greater affinity for antibodies directed against the N-terminal region of glucagon than towards C-terminally directed antibodies. Further analysis of the GLI by reverse phase high performance liquid chromatography revealed considerable molecular heterogeneity but no component with the elution volume or immunochemical properties of glucagon was identified. A major component, purified to homogeneity using octadecysilyl- and phenyl-silica columns, had the composition: [asx_6 thr_2 ser_5 glx_5 gly_4 ala_3 val ile leu_4 tyr_2 phe_2 his lys_3 arg_4 trp]. The absence of a methionine residue indicated that the peptide does not contain the full sequence of glucagon. The composition of this peptide resembles that of the intestinal peptide, glucagon-37 (glucagon.lys arg asn lys asn asn ile ala) more closely than that of glucagon. It is proposed, therefore, that the post-translational processing of proglucagon follows a different route in the guinea pig islet compared with the pathway in other mammalian species.

89. Diabetes in BB-rats is preceded by low levels of immune complexes despite normal IgG concentrations

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Circulating immune complexes (IC) are often found to be increased in recent-onset insulin-dependent diabetic patients. Since BB rats develop insulin-dependent diabetes similar to that in man, IC levels were determined from an early age until onset of diabetes. Samples collected on days 12, 22, 29, 43, 71, and 99 from 12 BB/Hagedorn (BB/H) and 12 BB control rats (non-diabetic w-subline) were analysed for IC, serum IgG and lymphocytes. Diabetes developed in 8 of 12 BB/H rats between 75–93 days. IC were assayed in a solid-phase C1q test, using incubation with rabbit anti-rat IgG before adding ^{125}I-protein A. All samples were tested in triplicate; the intra-assay coefficient of variation was 4%, and inter-assay 15%. Levels of IC, IgG and circulating lymphocytes varied with age and increased after day 29, reaching maxima at days 71–99. BB/H and BB control rats were similar with respect to IgG levels, but levels of IC were lower in the BB/H rats throughout the study period ($p < 0.01$–0.001). The BB/H rats were markedly lymphopenic during the entire study period. Thus, low levels of IC without altered serum IgG, and lymphopenia, are features of diabetes-susceptible BB rats by the age of 12 days.

90. Visual acuity as a screening test for diabetic maculopathy

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We have investigated the use of visual acuity (VA) as a screening test for diabetic maculopathy in an outpatient setting. The best corrected VA was recorded in standardised conditions for 160 randomly selected patients aged ≥ 50 years. 27% of patients had no VA previously recorded. Results differed from last recorded VA by ≥ one line in 52% of eyes and were better in 34%. An ophthalmologist examined 110 patients without knowledge of VA and his findings were combined with details of 50 patients already attending an eye clinic. True VA was ≤ 6/12 in either eye in 69 patients (35%). 89% of patients with subnormal acuity had clinically discernible eye disease compared with 23% of those with normal acuity (usually background retinopathy or early cataracts). 22% of patients with subnormal acuity had diabetic maculopathy requiring treatment compared with 2% of those with normal acuity. Only one patient examined had proliferative retinopathy, which was advanced (VA < 6/60 both eyes) despite treatment. To be of use in detecting diabetic maculopathy, visual acuity needs to be measured in standardised conditions, preferably by a trained observer.

91. Insulin receptor kinase activity in liver

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We studied the ability of lectin purified rat liver insulin receptor (IR) to mediate ^{32}P incorporation into tyrosine residues of the 95K β subunit (β) of the IR (autokinase) and histone (exogenous substrate). Insulin stimulated ^{32}P incorporation into β and histone by 3-8-fold (mean 5.4 and 3.9, respectively). The dose response curve was similar for β and histone with mean EC_{50} values of 4.0 and 3.6 ng/ml free insulin, respectively. Insulin binding was measured under the same conditions as phosphorylation, and the EC_{50} for phosphorylation occurred at a mean fractional IR occupancy of 0.21. We also studied the kinase activity of IR from rats whose diets were manipulated to produce alterations in insulin action. ^{32}P incorporation into β was comparable in control, fasted (48 and 72 h), and high fat and high carbohydrate-fed rats. In contrast, insulin stimulation of ^{32}P into histone was decreased by 25% using IR from fasted rats. If phosphorylated β represents the active kinase, then ^{32}P into the exogenous substrate histone should be expressed per phosphorylated β. At maximal insulin, ^{32}P incorporation into histone/pmol of phosphorylated IR was decreased by 16% in the fasting state.

92. Clotting factors and platelet function in Type 1 (insulin-dependent) diabetic patients with vascular disease after a short period of strict metabolic control

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We studied the effect of strict metabolic control on clotting factors (II, V, VII, X, XII, VIII-C, VIII-R:A, fibrinogen) and platelet function (β-thromboglobulin and platelet factor 4, PF4) in eight Type 1 diabetic patients (mean age 38 ± 8 years, duration of diabetes 13 ± 5 years), all with poor metabolic control and vascular complications. Patients were connected to an artificial pancreas (Biostator) for two 24-h successive periods; the first for glucose monitoring only, the second for feed-back control. Basal blood glucose was 13.8 ± 2.2; after monitoring 14.1 ± 2.3; after feed-back control 5 ± 0.3 mmol/l. With respect to a control group, in diabetic patients there were significantly higher levels of β-thromboglobulin (115 ± 32 ng/ml), PF4 (10.7 ± 2.1 ng/ml), FVII (121 ± 11%), FVIII-R:A (169 ± 41%), FVIII-C (133 ± 20%), fibrinogen (3.62 ± 0.9 g/l), with no significant modification at the end of the monitoring period. At the end of the feed-back period, there were significant reductions in β-thromboglobulin (68 ± 28, $p < 0.01$), PF4 (6.7 ± 2, $p < 0.01$), FVIII-C (98 ± 3, $p < 0.05$), fibrinogen (2.81 ± 0.6, $p < 0.05$) with a slight but not significant reduction of FVII and no modification of FVIII-R:A Consequently the hypercoagulable state, observed in diabetic patients with vascular complications, appears to be related to the metabolic derangement rather than to the vasculopathy and it can be for the most part corrected with improved metabolic control.

93. Cortical osteopenia and metatarsal fracture precede Charcot's osteoarthropathy

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Extensive bony disorganisation is characteristic of the Charcot joint. The development of such change is usually attributed to trauma to the painless foot. We have examined the possibility that abnormal bone metabolism may precede the overt manifestations of Charcot's osteoarthropathy. Cortical bone area (expressed as a proportion of total cross-sectional area) at the mid-point of the second metatarsal and metacarpal was measured on radiographs obtained from 41 diabetic patients. 19 patients with severe neuropathy were compared with a control group of 22 patients (matched for age, sex and duration of diabetes) with no evidence of neuropathy. Compared to controls the neuropathic patients had significantly less cortical bone in both metatarsals and metacarpals ($p < 0.0005$ and $p < 0.002$, respectively). Metatarsal fractures were noted in six of the patients with neuropathy but not in the control subjects ($p < 0.02$). In five of the six cases the fracture had been reported as painful and in all cases the foot had been hot and swollen, and fracture followed only minimal trauma. Two to 12 months subsequent to these studies five of the fracture cases went on to develop typical Charcot joints. We conclude that cortical osteopenia occurs in patients with neuropathy and predisposes to metatarsal fracture. Such fractures precede the development of Charcot's osteoarthropathy.

94. Metabolic effects of endurance- and strength-exercise training programmes in normal weight Type 2 (non-insulin-dependent) diabetes mellitus

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Metabolic effects of physical training were investigated in 20 normal weight sulphonylurea-treated Type 2 diabetic patients who were subjected either to an endurance training (ET; $n = 11$, mean age 46 years, mean duration of diabetes 8 years) or to a strength-exercise training programme (ST; $n = 9$, age 51 years, duration of diabetes 10 years) twice weekly for 2 h over a period of 3 months. During ET maximal oxygen capacity ($\dot{V}O_{2max}$) increased significantly, whereas ST resulted in an increase of muscular strength parameters only. Body weights remained constant during ET and ST. During ET fasting blood glucose fell from 8.2 ± 0.8 to 6.9 ± 0.8 mmol/l ($p < 0.05$), HbA_{Ic} from 8.8% to 7.2% ($p < 0.01$) and HDL-cholesterol rose from 2.1 ± 0.1 to 2.5 ± 0.1 mmol/l ($p < 0.05$). During ST fasting glycaemia fell from 9.6 ± 1.2 to 9.0 ± 1.0 mmol/l ($p < 0.05$), HbA_{Ic} from 9.5% to 8.2% ($p < 0.05$) and HDL-cholesterol rose from 0.96 ± 0.05 to 1.11 ± 0.08 mmol/l ($p < 0.05$). Both types of training led to a significant improvement of glucose tolerance following an oral 100 g glucose load. Physical training of 3 months' duration, resulting in measurable increases in physical fitness, was associated with substantial improvements of metabolic control in Type 2 normal weight diabetic patients, irrespective of whether endurance or strength exercise training was carried out.

95. Optimized normoglycaemic insulin replacement: a useful approach to treatment of Type 1 (insulin-dependent) diabetes

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Optimized normoglycaemic insulin replacement (ONIR) is achievable in Type 1 diabetes by continuous infusion or by multiple injection of insulin. The latter requires substitution of (1) basal insulin secretion by twice-daily injection of long-acting insulin, (2) prandial insulin need by pre-prandial regular insulin, and (3) blood glucose (BG) monitoring in combination with useful algorithms for correction of BG levels off the target range. To evaluate the applicability of ONIR, 56 Type 1 diabetic patients were included in the study; metabolic profiles of BG and free insulin and in part also of non-esterified fatty acids, β-hydroxybutyrate and lactate were determined before (1) one week and (2) 8 weeks (= ambulatory; 3) after switching the patients from conventional therapy to ONIR. Thereby mean BG was reduced from 11 ± 2.9 (1) to 5.9 ± 1.1 (2) and 7.2 ± 1.6 (3) mmol/l, and HbA_{1c} became normal ($5.5 \pm 0.7\%$) in 81% of the patients, of whom 70% also achieved aglycosuria. Computer-assisted evaluation of ambulatory BG measurements gave a mean value of 6.8 ± 1.2 mmol/l. Long-term follow-up (1720 patient-weeks) showed that ONIR (A) not only almost normalized BG but also was well accepted by the patients, (B) improved the patients' personal freedom by permitting them to influence directly BG, diet and insulin dose and thus appears (C) as a useful therapeutic option in treatment of Type 1 diabetes.

96. Rapid lowering of blood glucose may lead to cottonwool exudates in the retina: studies in Type 1 (insulin-dependent) diabetic patients treated with continuous subcutaneous insulin infusion and multiple insulin injections

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To study early retinopathy during intensified insulin treatment 45 C-peptide-negative diabetic patients (aged 18–42 years, duration of diabetes 7–22 years, without proliferative retinopathy) were followed prospectively for one year. After a 2-month pre-period they were randomly assigned to: (M) 15 received rapid insulin before each meal (4–6 × daily) and NPH insulin at bedtime; (P) 15 received continuous subcutaneous insulin infusion (CSII); (C) 15 received two daily injections. All groups improved blood glucose control in the pre-period ($p < 0.001$); P and M improved further to near-normoglycaemia ($p < 0.01$), but C was unchanged. Retinopathy was evaluated blind by 3-monthly fluorescen angiography and colour fundus photographs, and by counting cottonwool exudates (CWE) of the entire retina during eye examinations. 15 patients developed CWE after 3–6 months of treatment: seven patients on P, eight on M and no patients on C. CWE regressed in all but four patients after one year. Two-thirds of the patients were women. HbA_1 decreased from 11.6% for CWE-patients and 10.6% for non-CWE-patients (NS) to 8.3% and 9.3%, respectively after 3 months of treatment. CWE-patients decreased significantly more than non-CWE-patients: $\Delta HbA_1 = 3.3\%$ versus 1.3% ($p < 0.01$). This indicates that rapid lowering of blood glucose by means of both CSII and multiple insulin injections, may promote transient deterioration of retinopathy.

97. Epidemiology of diabetes in Swedish children aged 0–15 years: a 6-year prospective study

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Since 1 July 1977, all newly diagnosed diabetic children in Sweden have been reported to a central register. We report epidemiological data for the first 6 years covering 2,293 diabetic children in a total population of 1.6 million children 0–15 years of age. The degree of ascertainment in this study was 93%. Comparing the first and second 3-year periods, an increase in incidence rates was found (22.7–24.9/100,000). This increase by time was consistent when analysing incidence rates by age, sex and geographical distribution. The incidence was also increased compared to a smaller retrospective study in Sweden covering the same age groups 1970–1975 (19.6/100,000). Cumulative incidence rates revealed a risk of developing diabetes at age 15 years of 3.8‰ for boys and 3.5‰ for girls. The higher incidence for boys was consistent for all age groups. Incidence peaks were noted for both boys and girls in the pubertal ages. Seasonal variations were very consistent during the years studied, showing incidence peaks in August–October and December–January. Using life table methods, significant geographical variations within the country were shown. It was approximately twice as common that a first degree relative with insulin-dependent diabetes was a father than a mother.

98. Computerized mastery learning instruction of diabetic children

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To improve the knowledge of diabetes self-care in children we have adapted the mastery learning technique as follows: the programme comprises eight lessons on different aspects of self-care; for every lesson computerized units, booklets, audiovisuals, according to the programmed instruction, and computerized multiple choice tests for formative evaluation were developed; children were instructed individually (44 ± 1 min) and evaluated by computer (13 ± 0.6 min); then, they studied booklets at home according to the suggestions of formative evaluation and were re-evaluated by computer after 1 week; subjects with inadequate level of mastering were re-instructed by doctors either individually or in small groups. We report the results found in 30 children (aged 9–17 years; duration of diabetes 1–10 years) instructed on recognition, treatment and prevention of hypoglycaemic crisis. The mean percentage of knowledge achieved was: previous

traditional individual instruction (3 years) 48%; mastery learning (1 week): computer 82%, booklet 100%; evaluation of knowledge retention after 9 months: 98%. Instruction by computer was found not difficult (85%), interesting (100%), enjoyable (98%). Based on this approach, which appears more effective and motivating than traditional methods, a programme is in progress for educating all diabetic children in our region.

99. Ischaemic heart disease in occult and previously known Type 2 (non-insulin-dependent) diabetes

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The prevalence of ischaemic heart disease and the association with blood pressure were studied in 87 subjects with newly-diagnosed fasting hyperglycaemia, 228 previously known diabetics and 305 sex- and age-matched control subjects found during a screening for diabetes of 60–74-year-old inhabitants ($n = 5699$) in a Danish municipality (Fredericia). The diagnostic criterion for newly-diagnosed patients was one fasting blood glucose $\geqslant 7$ mmol/l. Heart disease was defined according to ECG changes at rest or during exercise. Women with newly-diagnosed diabetes had a significantly higher prevalence of heart disease (49%) than the corresponding non-diabetic women (27%; $p < 0.05$), whereas no difference was seen in men (53% and 51% respectively). In previously-known diabetic patients both men (69%) and women (51%) had a higher prevalence than the corresponding control groups (men: 49%, women: 38%; $p < 0.01$). In conclusion, an occult elevated fasting blood glucose level is associated with an increased prevalence of heart disease in women, whereas a longer duration of diabetes increases the prevalence in both sexes. No association was found between blood pressure and heart disease.

100. Fall in plasma glucose on storage of whole blood samples

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We have examined the effects of storage of whole blood on plasma glucose. Blood samples (4–17 mmol/l) were taken, stored in K EDTA/F^- tubes and assayed at various times thereafter by a glucose-oxidase continuous-flow system (coefficient of variation $< 0.7\%$ over this range). Plasma glucose fell from 9.86 ± 1.63 mmol/l (mean $\pm$ SEM) initially to 9.7 ± 1.62 after 30 min ($p < 0.01$) and to 9.49 ± 1.62 at 6 h ($p < 0.001$). The rate of fall was lower at 4 °C than 25 °C (0.47 ± 0.03 versus 0.29 ± 0.03 mmol/l at 6 h, $p < 0.05$) and was positively correlated with haematocrit ($r = 0.967$). Red cell intracellular glucose and total glucose were determined using ^{14}C-inulin to determine intracellular volume. Whereas plasma glucose fell over 6 h to $95.4 \pm 0.84\%$ of initial value, intracellular glucose, initially at $79.2 \pm 3.1\%$ of extracellular glucose, rose to $96.7 \pm 4.5\%$ over the same period, with the effect that total glucose remained unchanged at $100.4 \pm 3.4\%$ of initial values. We conclude that the small fall in plasma glucose seen on storage is due to the intracellular shift rather than incomplete fluoride inhibition of glycolysis. Samples should be centrifuged as soon as possible after collection, particularly in research situations where small falls may be significant.

101. Characterisation of an intermediate in the degradation of [Phe^{B1}-^{3}H] insulin by insulin protease

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[Phe^{B1}-^{3}H] insulin was degraded by insulin protease (E. C. 3.4.22.11) in vitro under conditions which gave about a 40% increase in the amount of radioactivity soluble in trichloroacetic acid. Gel filtration of the products on Sephadex G50 separated three peaks of radioactivity. The first eluted in the position of insulin, the second just after insulin and the third as small molecules. Control samples incubated without enzyme or with enzyme inhibited by N-ethylmaleimide gave only radioactivity eluting in the position of insulin. The peak of degraded material eluting just after intact insulin was characterised by its behaviour on electrophoresis after various chemical or enzymatic treatments. It had a reduced mobility on paper electrophoresis in 30% formic acid compared with insulin, as had its performate-oxidised form compared with oxidised insulin. Its mobility and the mobility of its oxidised form were not altered by treatment with trypsin or carboxypeptidase B, alone or sequentially, under conditions where the mobility of insulin is affected. These results are consistent with the cleavage of the insulin B chain in the regions B7 to B10 as well as between B19 and B22.

102. Direct comparison of K^+ and Rb^+ efflux in normal mouse islets

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Changes in potassium permeability are involved in controlling insulin release from β cells. Some previous studies of K^+ flux used $^{42}K^+$ but most used $^{86}Rb^+$. A unique series of double-labelled experiments have been performed, enabling an exact direct comparison of efflux rate constants. Collagenase-isolated mouse islets were loaded in the presence of 5 mmol/l glucose. One minute perifusate samples were collected and counted immediately and after $^{42}K^+$ had decayed. $^{42}K^+$ and $^{86}Rb^+$ efflux rate constants (k_K and k_{Rb}) were computed. Two types of experiment are reported: (1) Efflux in 0 glucose with alternate pulses of 50 mmol/l K_0^+ and 50 mmol/l Rb_0^+ (osmolality constant), that cause similar changes in membrane potential. (2) Efflux in the presence of glucose 0–22.2 mmol/l. The ratio k_{Rb}/k_K defines the permeability ratio independently of the membrane potential. The ratio in 0 glucose was 0.81 increasing to 0.91 in 50 mmol/l K_0^+ and to 1.06 in 50 mmol/l Rb_0^+. In the presence of glucose, the ratio was essentially unchanged. The data indicate that $^{86}Rb^+$ is a passable isotope for K^+ efflux. However, the variations in the ratio found in some situations may indicate that there is more than one type of channel with different Rb^+/K^+ selectivity ratios.

103. The dawn phenomenon in very young diabetic children

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The magnitude of the metabolic disturbance in very young diabetic children has been recognised but seldom studied. Small doses of insulin are absorbed quickly, and hence the 'dawn phenomenon' might be marked in these patients. Nine C-peptide negative toddlers (< 6 years) were admitted for one overnight metabolic profile, with full parental consent. Six were on twice daily insulin regimens, and doses and diet were not changed. Plasma free insulin declined overnight in all children (mean $\pm$ SEM) from 14.9 ± 2.2 mU/l at 19.30 h to 5.4 ± 0.8 mU/l at 06.30 h. Blood glucose (peak 11.7 ± 2.0 mmol/l at 20.30 h) declined to 6.2 ± 1.2 mmol/l at 04.30 h rising in all patients before breakfast (9.8 ± 1.5 mmol/l at 07.30 h) with a marked peak thereafter (16.2 ± 2.1 mmol/l at 09.30 h). A corresponding pattern was seen in blood lactate (0.45 ± 0.03 mmol/l at 05.30 h, 0.69 ± 0.04 mmol/l at 07.30 h). Markedly abnormal rises were seen in blood glycerol (at 04.30 h, 0.05 ± 0.00 mmol/l; at 07.30 h, 0.12 ± 0.01 mmol/l) and particularly blood 3-hydroxybutyrate (at 04.30 h, 0.24 ± 0.05 mmol/l; at 07.30 h, 0.99 ± 0.27 mmol/l).

We attempted to explore the time relationship between insulin and glucose concentrations by distribution free analysis, correlating successive changes. This suggested a long delay (2–6 h) between the insulin concentration and effect. In conclusion, diabetic toddlers on injection therapy show a clear deterioration in metabolic control in the hours before breakfast, associated with insulin deficiency.

104. Ontogenic rat development of liver insulin and glucagon receptors in plasma membranes and Golgi elements

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It has been reported that insulin administration to adult rats induces a loss of insulin receptors in plasma membranes (PM) and an increase in the Golgi fraction (GF). To evaluate this fact, we have studied the distribution of insulin and glucagon receptors in PM and GF during ontogenic development, where the secretory hormonal patterns change significantly. Purity of PM and GF membranes was verified with 5′-nucleotidase and galactosyltransferase, respectively and by electron microscopy. Circulating insulin levels were greater in fetuses and smaller in nursing rats, while glucagon concentrations were 441 ± 11, 978 ± 41 and 182 ± 18 pg/ml in fetal, nursing and adult rats, respectively. Insulin receptor concentrations were greater in PM of nursing than in fetal and adult rats, while, in GF, were smaller than in the other two groups. In addition, glucagon receptor number in PM was proportional to the age of the animals, while in GF it was inversely related to plasma glucagon levels. Kinetic properties and degradation of both hormones and their receptors did not account for differences observed in binding. These results suggest that the changes of circulating insulin and glucagon levels during rat development could modify the concentrations of their surface and intracellular receptors and therefore target-cell sensitivity.

105. Rapid variations in glucose uptake in vivo: evidence for a circadian rhythm in insulin action in man

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To assess whether insulin action depends on time of day, five normal subjects were studied clamping plasma glucose at 4.8 ± 0.1 mmol/l and plasma insulin at 18 ± 0.4 mU/l (insulin infusion rate 8 mU/m^2 per min) and determining hepatic glucose production (HGP) and glucose utilization (Rd) (3-^{3}H-glucose) on three occasions: study 1 from 10.00 to 22.00 h, study 2 from 18.00 to 06.00 h, study 3 from 02.00 to 14.00 h. Sleep, exercise and diet were kept constant prior to studies. In all three studies Rd increased between 0 and 3 h from basal of 11.9 ± 0.4 to 14.4 ± 1 μmol·kg^{-1}·min^{-1} (study 2), to 16.4 ± 1.9 (study 3), but to 22.6 ± 1.6 μmol·kg^{-1}·min^{-1} (study 1; $p < 0.01$). Between 3 and 12 h, Rd had a plateau at 14.8 ± 0.3 μmol·kg^{-1}·min^{-1} in study 2, whereas it decreased in study 1 (13.7 ± 0.9 μmol·kg^{-1}·min^{-1} at 12 h), but increased to 27.1 ± 2.5 μmol·kg^{-1}·min^{-1} at 7 h in study 3. Rd was identical on clock hours of overlap periods in studies 1–3 regardless of precedent duration of insulin infusion. Cosinor analysis showed a circadian rhythm of Rd with zenith between 11.00 and 12.00 h (acrophase $-171°$) and nadir between 23.00 and 24.00 h ($p < 0.01$). HGP was suppressed by 80–90% throughout the 24 h. In conclusion, insulin action undergoes a circadian rhythm in man, insulin sensitivity being 100% greater at noon compared with midnight.

106. Hepatic and peripheral insulin resistance following streptozotocin-induced insulin deficiency in the dog

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We tested the hypothesis that insulin resistance can evolve from a primary β-cell defect. Insulin-mediated glucose uptake (M) (insulin clamp ≃ 50 mU/l), hepatic glucose production (HGP), and glucose-stimulated insulin secretion (+4.2 mmol/l hyperglycaemic clamp) were measured in 10 dogs before and 6 weeks after streptozotocin-induced diabetes. Fasting plasma glucose increased from 5.8 ± 0.1 to 11.1 ± 1.3 mmol/l. During the hyperglycaemic clamp, insulin secretion was reduced by 75% in diabetic dogs ($p < 0.02$) and M decreased from 75.6 ± 5.6 to 26.1 ± 3.9 μmol·min^{-1}·kg^{-1} ($p < 0.001$). During the insulin clamp, basal HGP was higher in diabetic dogs (17.2 ± 2.8 versus 13.9 ± 1.1 μmol·min^{-1}·kg^{-1}) and failed to suppress (8.3 ± 1.7 versus 0.6 ± 1.7 μmol·min^{-1}·kg^{-1}, $p < 0.02$). Insulin-stimulated glucose clearance was reduced in diabetic animals (3.1 ± 0.8 versus 5.1 ± 0.7 ml·min^{-1}·kg^{-1}, $p < 0.02$). The impairment in glucose clearance correlated ($r = -0.87$, $p < 0.01$) with the reduction in insulin secretion (hyperglycaemic clamp). Since hyperglycaemia enhances glucose uptake, seven diabetic dogs received insulin clamps after lowering their fasting glucose to normal. Under strictly comparable conditions of euglycaemia and hyperinsulinaemia, diabetic dogs metabolized 50% ($p < 0.02$) less glucose than normal dogs (12.2 ± 1.1 versus 24.4 ± 4.4 μmol·min^{-1}·kg^{-1}). In conclusion, (1) insulin deficiency leads to the development of insulin resistance; (2) this resistance is present in the basal and insulin-stimulated state and involves both hepatic and peripheral tissues.

107. Plasma fibronectin in diabetic patients with micro- and macrovascular disease

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Increase of plasma fibronectin (PF), an α_2-glycoprotein released by the endothelial cells after vessel damage was previously related to the presence of diabetic retinopathy. The present study was aimed at comparing PF levels of normotensive diabetic patients with macrovascular disease (group 1), assessed by clinical, ECG and oscillographic criteria, or microangiopathy (group 2) diagnosed by detectable retinal lesions with fluorescein angiography, patients with both (group 3), diabetics with no vessel complications (group 4) and controls (group 5). Groups of patients were matched for age, sex, length of illness and HbA_1 concentrations. PF, measured by an immuno turbi dimetric assay in groups 2 and 3 insulin-treated diabetic patients (mean ± SD group 2 0.92 ± 0.2 μmol/l; $n = 25$ and group 3 0.97 ± 0.14 μmol/l; $n = 11$, respectively) was significantly higher than in group 1 (0.77 ± 0.11 μmol/l; $n = 6$), group 4 (0.72 ± 0.13 μmol/l; $n = 17$) and group 5 (0.69 ± 0.13 μmol/l; $n = 56$); $p < 0.01$. Similarly in the group of diabetics on sulphonylureas PF levels in group 2 (0.91 ± 0.15 μmol/l; $n = 28$) and group 3 (0.97 ± 0.17 μmol/l; $n = 12$) were higher than in group 1 (0.80 ± 0.14 μmol/l; $n = 23$), group 4 (0.75 ± 0.14 μmol/l; $n = 56$) and group 5; $p < 0.01$. Our data suggest that diabetic macroangiopathy is not associated with an increase in PF which appears to be a marker only of microangiopathy.

108. Increased clearance of ^{3}H-oestradiol from the plasma of the diabetic rat

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In an attempt to elucidate the reduced nuclear retention time and the decreased activity on protein synthesis of oestradiol observed in diabetic rat uterus reported recently, we studied the metabolism of ^{3}H-oestradiol ($E_2{}^3H$) in streptozotocin-induced diabetic rats after intraperitoneal injection of tracer amounts of the hormone. Diabetic rats accumulated 15 times more ether insoluble metabolites by 10 min compared with controls. The ether soluble fraction contained more oestrone and non-polar material, but less oestradiol in the diabetic rats. The plasma disappearance rates of all fractions were faster in the diabetic rats. For oestradiol itself, the half-life was two to three times shorter in the diabetic rats. A faster disappearance rate was also observed after intravenous injection of $E_2{}^3H$. The metabolic clearance rate, measured by constant intravenous infusion, was 1746 ± 326 and 1136 ± 347 ml/h in diabetic and control rats, respectively ($p < 0.001$). In conclusion, $E_2{}^3H$ was cleared much faster from the plasma in diabetic rats, resulting in an early accumulation of metabolites. The rapid disappearance of oestradiol may play a role in the shorter retention time of the oestradiol receptor complex in the nucleus and the reduced hormonal activity at the uterine level.

109. Evidence for multiple populations of insulin receptor antibodies with different activities in a patient with hypoglycaemia

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IgG from a patient with lupus nephritis, insulin resistance and hypoglycaemia was purified on DEAE cellulose and then subjected to affinity chromatography on a protein A-Sepharose column. Four protein peaks were eluted by a step gradient from 5.5 to 2.3. Three of the four peaks (pH 5.5, 3.3 and 2.3) inhibited ^{125}I-insulin binding to IM-9 cells and rat adipocytes; one peak (pH 4.3) was unable to inhibit insulin binding. One peak (pH 3.3) was able to inhibit ^{125}I-monoclonal antibody binding, but not the further three peaks. Two peaks (pH 5.5 and 3.3) showed stimulatory activity on glucose transport in rat adipocytes. pH 5.5 had a weak ability to inhibit insulin binding, but a strong potency to mimick insulin action; pH 3.3 had a strong ability to inhibit insulin binding but less potency to mimick insulin action. The ability of the four peaks to inhibit insulin binding and to stimulate AIB uptake in human fibroblasts was studied. Surprisingly, the pH 4.3 fraction demonstrated itself a strong inhibitor of insulin binding and the more potent stimulator of AIB uptake. These studies indicate, therefore, that multiple populations of antibodies to the insulin receptor are present and that there may be multiple antigenic sites on the insulin receptor. There are antibodies whose predominant activity is to block insulin binding and antibodies whose predominant activity is to mimick insulin action; these effects seem tissue specific.

110. Identification with a proinsulin specific radioimmunoassay of an insulinoma secreting primarily proinsulin

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Lack of a specific antibody has previously precluded direct (one-step) measurement of proinsulin (pI) by radioimmunoassay. In this study, an antibody was raised against biosynthetic human pI that showed less than 1% cross-reactivity with insulin and C-peptide. A sensitive assay ($IC_{50} = 0.16$ nmol/l; detection limit 0.01 nmol/l), not requiring prior separation of insulin and C-peptide, was developed using this antibody and ^{125}I-labelled pI. The response of serum pI to oral carbohydrate (100 g, Boehringer Dextro-OGT) was measured in six healthy subjects and in a patient with malignant insulinoma and liver metastases who maintained serum glucose in the normal range (3.0–6.2 nmol/l) over a 48 h fast, despite elevated immunoreactive insulin (IRI) (103.6 ± 3.9 mU/l). In the healthy subjects, serum pI increased over basal levels (0.013 ± 0.001 nmol/l; mean ± SEM) by 0.024 ± 0.004 nmol/l ($p < 0.02$). The fasting pI/IRI ratio ($0.233 \pm$

0.027) fell to 0.084 ± 0.007 ($p < 0.001$) following carbohydrate. In the insulinoma patient, the fasting pI (0.638 ± 0.053 nmol/l) and rise in pI (0.520 nmol/l) were higher than in healthy subjects ($p < 0.001$) and the pI/IRI ratio did not change on stimulation (basal 1.129 ± 0.106; stimulated 1.086 ± 0.123). In conclusion, a one-step radioimmunoassay for proinsulin has been developed and used to identify an insulinoma secreting primarily proinsulin. This observation explains the lack of fasting hypoglycaemia in the patient despite high IRI levels.

111. Development of diabetes in the BB/E rat (II): expression of class II molecules (Ia) in islet cells

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In a longitudinal study, BB/E rats (the majority without overt diabetes) were killed at 30, 45, 60, 75, 90 and 105 days of age and the pancreas from 16 rats at each time point were snap-frozen. Cryostat sections (4 µm thickness) were screened for the presence of Ia (rat equivalent of human HLA-DR) and lymphocytic infiltration using monoclonal antibodies kindly donated by Dr D Mason (Oxford). Double-fluorochrome techniques were employed to identify individual endocrine cell types in the islets. In animals without overt diabetes Ia expression was minimal around the islets in most of the pancreases examined and only a few capillary cells in the exocrine tissue and occasional endothelial cells in the periphery of the islets were Ia positive. In contrast, the pancreases from rats with overt diabetes showed Ia expression on lymphocytes emanating from ducts and vessels and penetrating islet areas, and a marked increase of Ia positivity on capillary endothelial cells and interstitial tissues in and around islets. In islets where the majority of β cells no longer contained insulin, Ia was demonstrated in the cytoplasm of some of the remaining proinsulin/insulin-positive cells.

112. Glomerulosclerosis irrelevant to clinical nephropathy

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We studied kidney biopsies and renal function in 44 Type I (insulin-dependent) diabetic patients and 10 non-diabetic subjects. Seven diabetic patients had no clinical nephropathy (no proteinuria, blood pressure 133/80 mmHg, serum creatinine 90 µmol/l), 22 had early clinical nephropathy (persistent proteinuria, blood pressure 132/88 mmHg, serum creatinine 91 µmol/l, glomerular filtration rate (GFR) 111 ml/min per 1.73 m^2) and 15 had late stage clinical nephropathy (persistent proteinuria, blood pressure 156/97 mmHg, serum creatinine 177 µmol/l) with reduced GFR (52 ml/min per 1.73 m^2). Mean duration of diabetes was 20.1, 14.7, and 19.1 years, respectively. The renal tissue was examined by morphometric light microscopy using a point counting technique with its origin unknown to the reader. All biopsies from diabetic patients showed glomerulosclerosis. However, no significant morphological differences were found between biopsies taken from long-term diabetic patients without clinical nephropathy and patients with early clinical nephropathy, the relative area of the glomeruli occupied by mesangium being 18.1 ± 11.7% and 19.9 ± 7.6%, respectively, against 8.8 ± 4.3% in control subjects. Biopsies from patients with late stage nephropathy showed significantly increased mesangium and decreased area of open capillaries compared with patients without clinical nephropathy. In conclusion, the renal changes seen by light microscopy are not responsible for the development of early clinical nephropathy, but when clinical nephropathy has developed, severe glomerular changes will be superimposed on glomerulosclerosis.

113. Is ω-oxidation of long-chain fatty acids enhanced in diabetes?

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ω-oxidation is an alternative pathway for long-chain fatty acid catabolism which normally proceeds via β-oxidation. The end products of ω-oxidation are dicarboxylic acids (e.g. adipic and suberic acids). Plasma was assayed for adipic, suberic and β-hydroxybutyric acid by capillary column gas liquid chromatography in ketoacidotic diabetic patients (n = 4) and in a further group of insulin-dependent diabetic patients (n = 4) before and after optimal glucose control by 2 weeks of continuous subcutaneous insulin infusion (CSII) with human insulin (Eli Lilly). Mean plasma adipic acid in the patients with ketoacidosis was 31 µmol/l (normal range 1–3 µmol/l) and suberic acid 17 µmol/l (normal range 1–4 µmol/l). These levels returned to normal after therapy. There was no correlation with β-hydroxybutyric acid levels. In the CSII group, mean fasting adipic acid was raised (13.5 µmol/l) and returned to normal after CSII. Pump therapy abolished an abnormal prandial rise observed in plasma adipic acid (11 versus 0.25 µmol/l). Increased levels of ω-oxidation products have been demonstrated in the plasma of diabetic patients and their relationship to poor glucose control and ketoacidosis established. Levels return to normal on correction of ketoacidosis and improved control.

114. Hyperglucagonaemia induces insulin resistance

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Glucose and amino-acid metabolism were studied in seven healthy volunteers (23 ± 3 years) before and after a 72-h glucagon infusion (0.86 pmol · kg^{-1} · min^{-1}). Multiple euglycaemic insulin clamps (0.5, 1.0, 5.0 mU · kg^{-1} · min^{-1}) were performed with ^{3}H-3-glucose and continuous indirect calorimetry. Fasting plasma glucose (4.6 ± 0.1 versus 5.2 ± 0.2 mmol/l; $p < 0.05$), hepatic glucose production (HGP: 10.6 ± 0.6 versus 12.8 ± 0.6 µmol · kg^{-1} · min^{-1}; $p < 0.01$), and plasma insulin (10 ± 2 versus 15 ± 3 mU/l; $p = 0.06$) were higher after glucagon. Basal non-protein respiratory quotient (NPRQ) and glucose oxidation increased after glucagon ($p < 0.01$). Glucose metabolism decreased after glucagon at each insulin clamp level (26 versus 23; 47 versus 39; 71 versus 65 µmol · kg^{-1} · min^{-1}; $p < 0.01$) primarily due to reduced non-oxidative glucose metabolism (16 versus 8; 36 versus 24; 56 versus 48 µmol · kg^{-1} · min^{-1}; $p < 0.02$) while glucose oxidation increased. Following glucagon, NPRQ was > 1.00 during the two higher insulin clamps ($p < 0.01$ versus pre-glucagon) indicating net lipid synthesis. HGP was incompletely suppressed after glucagon at the lower dose insulin clamp (0.6 versus 3.3 µmol · kg^{-1} · min^{-1}; $p < 0.05$). Basal levels of gluconeogenic amino-acids decreased after glucagon; branched chain amino-acids were unchanged. Before glucagon, hyperinsulinaemia had no effect on alanine levels. Post-glucagon, alanine decreased by 7, 15, and 26% ($p < 0.01$) during the three insulin clamps. In conclusion, prolonged hyperglucagonaemia (a) causes an increase in fasting glucose levels and a sustained elevation in HGP, (b) induces peripheral and hepatic insulin resistance due to impaired non-oxidative glucose metabolism, (c) enhances lipogenesis, (d) lowers basal plasma gluconeogenic amino-acid levels, (e) unmasks a hypoalaninaemic effect of insulin.

115. Specific binding sites for oxyntomodulin (glucagon-37) in fundic glands isolated from the rat stomach

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Oxyntomodulin (glucagon-37), a 37-amino-acid peptide isolated from porcine jejuno-ileum, displays a tissue specificity different from that of glucagon. Its main site of action is the acid-secreting portion of the stomach both in vitro and in vivo, where this peptide is a powerful inhibitor of gastric acid secretion. To determine whether this biological action is mediated by specific binding sites at the level of the acid-secreting fundic glands, we studied their possible presence in isolated glands. A specific binding of mono^{125}I-oxyntomodulin, which was linearly correlated with the amount of glands added, was observed at 20 °C. This binding was inhibited by increasing concentrations of oxyntomodulin ($ED_{50} \simeq 10$ nmol/l) and by glucagon ($ED_{50} \simeq$ 100 nmol/l). Scatchard transformation of the binding data was shown to be curvilinear, with the following K_d and capacity for the high affinity sites: 2.4 nmol/l and 100 fmol/mg protein for oxyntomodulin and 27 nmol/l for glucagon. No noticeable effect of pentagastrin (40 µmol/l), histamine (2.5 mmol/l) or carbamylcholine (40 mmol/l) was observed. This is the first report on the presence of specific binding sites for oxyntomodulin. The characteristics of these sites in gastric glands indicate that oxyntomodulin acts directly on acid secretion through these specific binding sites.

116. Type 2 (non-insulin-dependent) diabetes care in a rural area and at a diabetes centre

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To evaluate quality of care for Type 2 diabetes metabolic control and late diabetic complications were studied in patients of similar age in a rural area (RA, $n = 549$) and at a diabetes centre (DC, $n = 511$). Major differences were seen in the mode of treatment (DC/RA: insulin 34/18%; sulphonylurea 25/58%; biguanides 17/2%; diet only 24/22%) and in the patients' body weights (Broca > 105% DC/RA:

56/68%). The percentage of patients displaying an abnormal metabolic state (blood glucose >8.9 mmol/l; $HbA_{1c} > 5.8\%$; glycosuria $>$ 3.5 g/24 h) and the presence of late complications was high for those treated with oral anti-diabetic drugs (DC/RA: blood glucose 47/55%; glycosuria 47/33%; HbA_{1c} 72/73%; HDL-cholesterol 32/63%; proteinuria 5/3%; peripheral neuropathy [PNP] 13.4/12.2; macroangiopathy 32/30%; retinopathy 22/22%) and also for those receiving insulin (DC/RA: blood glucose 79/78%; glycosuria 75/71%; HbA_{1c} 85/90%; HDL-cholesterol 6/63%; proteinuria 12/14%; peripheral neuropathy 29/49%; macroangiopathy 42/52%; retinopathy 48/55%). However, as mean duration of diabetes was shorter in the patients from the rural area than in those from the metropolitan area for both patients treated with oral anti-diabetic drugs (DC/RA: 8.5/6.3 years) and patients receiving insulin (DC/RA: 14.5/12.5 years), it appears that late complications occur prematurely in a rural setting. We conclude that poor therapeutic efficacy prevails in patients with Type 2 diabetes and new ways need to be explored to improve this situation.

117. Activated T lymphocytes in normal and diabetic pregnancy

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T-cell subsets and activated T cells were evaluated in 17 samples from normal pregnant women and 24 from pregnant diabetic patients. Total T cells, helper/inducer (T_h) and cytotoxic/suppressor (T_s) T lymphocytes were studied with the monoclonal antibodies OKT3, OKT4 and UCHT4 respectively. Monoclonal antibodies against class II surface antigens (L243, DA6.231, DA6.164) and against a 120K surface glycoprotein (4F2) were used to detect activated T cells. An increase in the ratio Th/Ts was observed in pregnant diabetic women, in contrast to normal women ($p < 0.05$). A clear increase in 4F2-positive lymphocytes both in normal (13 out of 17) and in diabetic pregnancies (17 out of 24) was found. The modifications in the number of class II-positive T cells did not reach statistical significance. These results suggest, in keeping with current theory, that pregnancy represents a state of immunoactivation in which a population of alerted but not fully activated immunocompetent cells seems to be increased. In diabetic pregnancy T-cell subsets are simultaneously influenced by phenomena related to diabetes and to pregnancy.

118. Significance of fetal distress identified during antepartum fetal monitoring in insulin-dependent diabetic pregnancies

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To minimize unexplained stillbirths in insulin-dependent diabetic pregnancies, fetal well being was assessed by antepartum monitoring while awaiting development of pulmonary maturity. Antepartum monitoring consisted of outpatient non-stress tests beginning at 32 weeks gestation. Fetuses with non-reactive non-stress tests were evaluated further by oxytocin challenge tests and/or biophysical profiles, and were delivered if fetal distress was diagnosed. Using this system, there were no unexplained stillbirths during management of 172 pregnancies over a 6-year period. Of 14 infants delivered because of fetal distress identified during antepartum monitoring, six were found to have major disorders (two congenital heart defects, myelomeningocele, syringomelia, microcephaly, and hydrops); while the other eight were subsequently discharged from the nursery an average of 8 ± 3 days after delivery without major residual neonatal morbidity. Thus this system of antepartum fetal surveillance: (1) eliminated unexplained stillbirths; (2) identified a subgroup of insulin-dependent diabetic pregnancies with a high rate of major fetal abnormalities; and (3) allowed for identification and subsequent timely delivery of the other distressed fetuses which had a high risk for developing neonatal morbidity and/or mortality, such that potential long-term adverse outcomes were avoided.

119. Effects of ciglitazone, a novel antidiabetic agent, on the pancreatic islets and kidneys of obese, insulin-resistant C57BL/KsJ-db/db mice

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The purpose of this study was to evaluate the morphology of pancreatic islets, renal glomeruli and tubules of db/db mice after chronic administration of ciglitazone. Pancreatic tissue was prepared for light (LM) and electron (EM) microscopy. The renal cortex was processed for LM evaluation of the deposition of IgG, IgM and PAS-positive material in the glomeruli and tubules. Under LM, the percentage of granulated islets was greater ($p < 0.01$) and disruption of islets by acinar cells was decreased ($p < 0.05$) in treated animals. LM morphometry indicated that islet number was greater ($p < 0.006$) in treated mice. Under EM, most β cells of treated mice were granulated and showed minimal stress. β cells of control mice were degranulated and displayed expansion of Golgi and rough endoplasmic reticulum. A reduction in IgM ($p < 0.01$) and IgG ($p < 0.01$) was observed in the glomeruli of treated mice. Moderate deposition of glomerular PAS-positive material was similar in treated and control mice. The tubular cells of treated mice were unremarkable whereas those of controls displayed glycogen vacuolization. In conclusion, ciglitazone promoted regranulation of β cells, efficient synthesis and storage of insulin, preservation of islet viability and integrity and retardation of some renal complications in db/db mice.

120. Effect of experimental hyperthyroidism on insulin action and secretion in man

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To assess the mechanism by which hyperthyroidism impairs carbohydrate homeostasis, insulin secretion, and dose-response effects of insulin (0.5, 1.0 and $10\ mU \cdot kg^{-1} \cdot min^{-1}$, glucose clamp technique) on glucose production, utilization (3-^{3}H glucose), oxidation (indirect calorimetry) and monocyte insulin binding were determined before and after administration of triiodothyronine (T_3, 150 µg/day for 14 days) in 10 normal volunteers. Plasma T_3 increased to hyperthyroid levels (from 125 ± 5 to 507 ± 36 ng/dl). Fasting plasma glucose (5.2 ± 0.1 versus 5.6 ± 0.1 mmol/l, $p < 0.005$) and insulin (7 ± 1 versus 10 ± 1 mU/l, $p < 0.01$) both increased. Despite increased plasma insulin responses (388 ± 108 versus $540 \pm 112\ mU \cdot l^{-1} \cdot min^{-1}$, $p < 0.01$), intravenous glucose tolerance was unaltered. Basal glucose production and utilization increased (2.01 ± 0.05 versus 2.45 ± 0.09; 2.02 ± 0.06 versus $2.41 \pm 0.07\ mg \cdot kg^{-1} \cdot min^{-1}$, respectively, $p < 0.0005$); suppression of glucose production by insulin was impaired after T_3 (K_m 22 ± 3 versus 37 ± 7 mU/l, $p < 0.05$). Maximal glucose utilization increased (13 ± 0.9 versus $10.65 \pm 0.6\ mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.0005$) without a change in K_m due to increased glucose oxidation (5.41 ± 0.61 versus $3.41 \pm 0.29\ mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.0005$). Insulin clearance was significantly increased at all infusion rates. Since monocyte insulin binding increased (10.5 ± 1 versus $7.40 \pm 0.5\%$ per 10^7 cells, $p < 0.02$), we conclude that T_3 excess causes insulin resistance by selectively impairing suppression of hepatic glucose production by insulin and that this occurs at a post-binding site.

121. Withdrawn

122. Effect of high cereal, low sodium and low fat diet on cardiovascular risk factors in mildly hypertensive diabetic subjects

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Recent dietary recommendations for diabetic patients include increased dietary carbohydrate and fibre with reduction in fat intake. It has been reported that high carbohydrate diets may be hypertriglyceridaemic and reduce HDL-cholesterol levels, thereby increasing cardiovascular risk. We have therefore studied serum lipid levels during a 3 month controlled trial of a high-fibre (40 g/day), high-carbohydrate (65% daily energy), low fat (15% daily energy) and low-sodium (50 mmol/day) diet in 25 mildly hypertensive diabetic patients (age 56.6 ± 7.2 years; ideal body weight $132.2 \pm 22.3\%$, 14 females, 11 males) compared with well-matched control subjects ($n = 25$). Dietary therapy reduced serum triglyceride (1.8 ± 0.9 to 1.5 ± 0.8 mol/l, $p < 0.05$) glycosylated haemoglobin (12.4 ± 3.1 to $10.5 \pm 2.9\%$; $p < 0.001$) and weight (74.6 ± 13.5 to 71.7 ± 12.1 kg; $p < 0.01$), while HDL_2 increased (0.3 ± 0.2 versus 0.5 ± 0.3 mol/l; $p < 0.05$). Serum cholesterol and HDL-cholesterol remained unchanged. In hyperlipidaemic subjects (serum cholesterol >7.1 mol/l; triglyceride >2.1 mol/l) dietary treatment reduced cholesterol ($p < 0.02$) triglyceride ($p < 0.01$) glycosylated haemoglobin ($p < 0.01$) and weight ($p < 0.05$). In these 50 diabetic patients significant correlations were found between reduction in glycosylated haemoglobin and triglyceride ($r = +0.48$, $p < 0.001$) and cholesterol ($r = +0.36$, $p < 0.005$). Weight loss correlated only

with reduction of triglyceride ($r = +0.25$, $p < 0.04$). We conclude this dietary regimen is not hypertriglyceridaemic, but improves several cardiovascular risk factors and further supports the current dietary recommendations for diabetic subjects.

123. Retinal exudates in comparison to haemorrhages occur in patients with less hyperglycaemia and cholesterol ester linoleate

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The retinae of 137 patients were examined ophthalmoscopically and for visual acuity at diagnosis of non-insulin-dependent diabetes and again in 1982 and 1983, approximately 7 and 8 years later, when colour photographs were also taken. In 1983, 46% were without detectable retinopathy, 32% had haemorrhages (including microaneurysms) only, 4% exudates alone and 18% both lesions. Those with haemorrhages were more hyperglycaemic than those with exudates as judged by higher mean fasting plasma glucose (0, 1, 3 and 5 years from diagnosis) (9.7 versus 6.8 mmol/l, $p < 0.01$) and higher glycosylated haemoglobin in 1982. Indeed, those with exudates alone had lower mean glucose levels than those without retinopathy ($p < 0.05$). These relationships probably explain why haemorrhagic retinopathy was unduly associated with treatment with sulphonylureas ($p < 0.025$). Patients with exudates alone tended ($p < 0.075$) to be older than those with haemorrhages. Patients with exudates (± haemorrhages) had lower percentage of the fatty acids of the plasma cholesterol esters as linoleate (determined by gas chromatography) than those without ($p < 0.05$), but this did not hold for those developing haemorrhages. However, haemorrhages were associated with a higher percentage of arachidonic acid in these esters ($p < 0.001$). In some respects, exudate formation is more akin to macro- than microangiopathy.

124. A unique mechanism in paracrine regulation: role of translocation of somatostatin receptors during exocytosis

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We have demonstrated that glucose-induced insulin release in isolated islets is accompanied by enhancement in somatostatin binding. In this study, we have correlated the margination of somatostatin receptors to the plasma membrane with the ability of somatostatin to inhibit insulin release. Islets were perifused with increasing concentrations of glucose in the presence of sodium isethionate (NaIs). NaIs inhibits insulin release, but not the recruitment of somatostatin receptors. The margination of secretion vesicles to the surface membrane continued without their lysis. Somatostatin binding rose from 1.5 ± 0.2 to 3.9 ± 0.5 fmol/10 islets as glucose concentration increased while insulin release was unchanged. NaIs was then removed and islets challenged with 3-isobutyl-1-methyl-xanthine (IBMX) and somatostatin. The inhibition of IBMX-induced insulin release by somatostatin was proportional to somatostatin binding. Somatostatin reduced insulin release by 50 μU/100 islets initially perifused with low glucose and by 585 μU/100 islets perifused with high glucose. We conclude: (1) glucose enhances translocation of somatostatin receptors to the surface of pancreatic islets in a dose-dependent manner; (2) inhibition of insulin release by somatostatin is proportional to somatostatin binding; and (3) translocation of somatostatin receptors during exocytosis plays an important role in paracrine regulation of insulin release by rendering islets more sensitive to somatostatin.

125. Effects of 1,3-bis(2-chloro-ethyl)-1-nitrosourea on glutathione reductase activity and insulin release in pancreatic islets

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In pancreatic islets exposed to nutrient secretagogues, an increase in the tissue content of thiol groups may participate in the stimulation of insulin release. We investigated, therefore, the effects of 1,3-bis(2-chloro-ethyl)-1-nitrosourea (BCNU), an inhibitor of glutathione reductase, on metabolic, ionic and secretory events in rat islets. BCNU caused a dose-related (0.1–1.0 mmol/l) and time-related (15–60 min) decrease in glutathione reductase activity. This coincided with a fall in both the glutathione thiol:disulphide ratio and thiol group content. After 60-min pre-incubation in the presence of BCNU (0.5 mmol/l), D-(U-^{14}C)glucose oxidation, as well as glucose-stimulated ^{45}Ca net uptake and insulin release, were inhibited. The oxidation of L-(U-^{14}C)leucine and L-(U-^{14}C)glutamine was unaffected, however. Yet, the secretory response to these two amino-acids (10 mmol/l each) was severely impaired, this being associated with a modest decrease in ^{45}Ca net uptake. The release of insulin evoked, in the absence of Ca^{2+}, by Ba^{2+} and theophylline was unaffected by BCNU. These findings suggest that the thiol:disulphide balance of endogenous proteins depends on the integrity of glutathione reductase and participates in the secretory sequence at a site proximal to the activation of exocytosis by divalent cations.

126. Time course of glucose-induced changes in [^{3}H] arachidonic acid labelled phospholipid and endogenous Ca^2 ionophores in neonatal rat pancreatic islets

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In cultured islets labelled with [^{3}H] arachidonic acid (AA), glucose stimulation (16.7 mmol/l, 1–15 min) prompted a sustained fall in [^{3}H] AA content of phosphatidylinositol and polyphosphoinositides (57% and 59%, respectively of non-stimulated value by 15 min) and a concomitant transient rise in 1,2 diacylglycerol (DAG) and phosphatidic acid (PA) (250% and 500%, respectively at 2 min). Subsequently [^{3}H]AA content of DAG and PA fell and AA metabolites were detected in islet incubation medium. When lipid species with $^{45}Ca^{2+}$ transporting ability were examined in a model system a transient fall in plasma membrane-associated activity was followed by an increase in total islet ionophoretic activity. Formation of PA, AA metabolites and ionophoretic lipids was not affected by removal of extracellular Ca^{2+} but was reduced in the presence of 8-(N,N-diethylamine)-octyl 3,4,5 trimethoxybenzoate HCl (TMB8). Incubation of homogenates of glucose-stimulated islets with PI specifically labelled with AA in the 2-position acyl chain and [^{3}H] arachidonyl PA, generated AA in a calcium-dependent manner. These findings demonstrate both phospholipase C and A_2 actions following glucose and indicate the temporal relationship of products of glucose-induced phospholipid turnover which may affect islet Ca^{2+}availability, endogenous kinase activation and eicosanoid synthesis.

127. Studies on the biological activity of fragments of β-cell-tropin, $ACTH_{22-39}$, in the perfused rat pancreas

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β-Cell-tropin (β-CT) is a potent insulin secretagogue released by the pituitary neurointermediate lobe of genetically obese mice; it has been characterized as $ACTH_{22-39}$. A rapid monophasic stimulation of insulin release in the perfused rat pancreas has been demonstrated with 0.1 nmol/l of the peptide in the perifusate. A hexapeptide corresponding to β-CT_{1-6} ($ACTH_{22-27}$) (synthesized by Dr. J. Humphries, Beecham Pharmaceutical Research Division) has no biological activity at a concentration of 3 nmol/l. A C-terminal peptide β-CT_{6-18} ($ACTH_{27-39}$ – donated by Dr. B. Riniker at Ciba-Geigy) is active at 1.5 and 15 nmol/l. β-CT itself and β-CT_{6-18} are being tested at a range of concentrations to determine whether the shorter peptide has the full biological potency of the complete pituitary insulin secretagogue. When the minimal effective concentration of β-CT has been established this will be used as a standard to compare the relative activities of smaller fragments, to be prepared either synthetically or by aminopeptidase and carboxypeptidase degradation of β-CT and subsequent purification on reverse-phase high performance liquid chromatography.

128. Diabetes in the BB-rat is preceded by neonatal lymphopenia and is not affected by cortisone treatment

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The appearance of islet-cell-surface antibodies (ICSA) and lymphopenia in spontaneously diabetic BB-rats was determined in three groups of rats studied between 12–140 days of age: 27 diabetes-prone BB/Hagedorn rats, 23 BB-control rats (non-diabetic w-subline) and 18 Wistar rats. Circulating lymphocytes were determined weekly in the first month and then biweekly. No differences were found in lymphocyte concentrations between BB-controls and Wistars, either on day 12 (3474 ± 1275 versus 3369 ± 1610, mean ± SD, respectively) or in the relative increase in lymphocytes until steady state (4.4 versus 4.7 times that at day 12). In contrast, BB/H showed lymphopenia by day 12 (2318 ± 1358, $p < 0.005$ versus BB-controls) and a relative increase to steady state of only 2.8 times. The effect of early immune-intervention was studied in 40 diabetes-prone BB-rats treated with cortisone acetate (0.75 mg intraperitoneally every third day) and 40 matched BB-rats with saline from 21 days of age. ICSA was followed

from 55 days of age. Cortisone treatment did not affect the incidence of ICSA (17/40 versus 15/40 in controls), diabetes (21/40 versus 18/40 respectively) or degree of lymphopenia in diabetics (3807/μl versus 4028/μl (mean values)). In conclusion, clinical diabetes in BB-rats is preceded by neonatal lymphopenia. Cortisone therapy from early life does not affect appearance of ICSA, lymphopenia or the incidence of diabetes.

129. Insulin resistance in the heart: characterization of receptor and post-receptor defects in isolated cardiocytes of genetically obese Zucker rats

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The role of cardiac muscle in the abnormal glucose handling observed in obesity is unclear. Isolated cardiac myocytes from Zucker rats were used here to characterize the defective sites related to this syndrome. Scatchard analysis of insulin binding data suggested a reduction in the number of low-affinity sites in cells from obese rats; moreover, insulin internalization was found to be decreased by 70% in these animals. Bacitracin increased insulin binding (40%) and decreased insulin internalization (50%) only in the lean group, though inhibiting insulin degradation equally in both groups. The maximal velocity of the glucose carrier was reduced by 45% in obese animals. Glucose transport exhibited unaltered sensitivity to stimulation by insulin but decreased responsiveness (42%) in cardiocytes from obese rats. Treatment of cells with the hypoglycaemic compound POCA resulted in an increase of glucose transport in obese rats without normalization of insulin responsiveness. In conclusion, our data show that insulin resistance in cardiac muscle of obese Zucker rats is related to defects at both receptor and post-receptor levels. The former involves decreased insulin binding and altered insulin processing; the latter involves decreased glucose transport activity, but appears unrelated to increased fatty acid oxidation.

130. Reduction in the number of major and minor limb amputations; impact of a new combined diabetic foot clinic

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Diabetic foot ulceration remains a major problem. However, in the combined foot clinic set up in May 1981 we have achieved healing in 180 out of 205 neuropathic ulcers and 87 out of 125 ischaemic ulcers, thus reducing the total number of foot operations per year from 39 ± 2.8 to 21 ± 5.8 ($p < 0.01$) and major amputations from 11 ± 1.4 to 6 ± 1.0 yearly ($p < 0.01$). Since its inception, 81% of wage-earners have continued at work throughout treatment, with only 24 hospital admissions per year. The clinic has brought together for the first time the skills of chiropodist, physician and shoe-fitter. It provides intensive chiropody (patients attending every 3.3 ± 1.5 weeks during ulceration and every 7.5 ± 1.4 weeks after healing), precise antibiotic therapy to eradicate staphylococcus aureus (45% of lesions) and streptococcus pyogenes (11% of lesions) and specially constructed shoes to hasten healing and prevent relapse. Mean time of healing was 9.7 ± 8 weeks for neuropathic ulcers and 13.1 ± 9 weeks for ischaemic ulcers. Relapse rate in surgical shoes was 19% compared with 91% ($p < 0.01$) in patients who preferred their own shoes. We believe the combined diabetic foot clinic is a major advance in the care of both the neuropathic and the ischaemic foot.

131. Prednisolone and betamethasone have different effects on insulin resistance in patients with Type 2 (non-insulin-dependent) diabetes

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In a group of Type 2 diabetic patients the effects of 3 days' administration of either betamethasone (0.5 mg three times a day) or prednisolone (5 mg three times a day) on glucose metabolism and insulin resistance were investigated using ^{3}H-3-glucose as tracer. We found that both glucocorticoids increased 24-h blood glucose without changing fasting plasma glucose values. Fasting plasma insulin and C-peptide increased significantly after betamethasone (insulin 34.8 ± 20.4 to 78.3 ± 32.7 pmol/l; C-peptide 350 ± 230 to 550 ± 240 pmol/l), but not after prednisolone. Fasting plasma glucagon remained unchanged during treatment with both glucocorticoids. After an insulin bolus (0.1 U/kg body weight) fasting plasma glucose fell at the same rate in both the control and the glucocorticoid experiments. The glucocorticoids did not influence glucose turnover, either in fasting steady state conditions, or after the insulin bolus. It is concluded that in the dose administered only betamethasone induced insulin resistance. It is suggested that the difference in the action of the two glucocorticoids is caused by different effects on cellular insulin receptors.

132. Insulin release and sensitivity as predictive factors in the development of glucose intolerance and Type 2 (non-insulin-dependent) diabetes

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From a group of 226 subjects, in which an oral glucose tolerance test (GTT), insulin response to glucose infusion test (GIT) and insulin sensitivity had been determined at the onset, 163 were retested by an oral GTT 5–8 years later. They included the following subjects listed according to their original oral GTT: 116 normal, 33 borderline and 14 decreased. Insulin response to GIT and insulin sensitivity were determined by parameter identification in a mathematical model. Out of the 116 normal subjects, ten had developed decreased glucose tolerance and one manifest diabetes; nine of these originally demonstrated either low insulin response in relation to insulin sensitivity or marked insulin resistance. Out of the 33 with a borderline oral GTT, two developed manifest diabetes and one decreased glucose tolerance all originally with low insulin response. Out of 14 subjects with an initially decreased oral GTT, eight developed manifest diabetes. They all originally showed severely impaired insulin release on GIT. None with borderline or decreased glucose tolerance and high insulin response developed diabetes. We conclude that low insulin response in relation to prevailing insulin sensitivity is an early marker of Type 2 diabetes. A decreased oral GTT in combination with exaggerated insulin response is not related to Type 2 diabetes but represents a separate metabolic entity.

133. Sympathetic regulation of lipid mobilization during prolonged fasting in man

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To examine the influence of prolonged fasting on the sympathetic regulation of lipid mobilization: (1) the effects of adrenergic agonists on lipolysis, and (2) the specific binding of labelled adrenergic antagonists were investigated in isolated fat cells. The study was performed in 17 women with non-endocrine obesity. Subcutaneous fat specimens were removed from the hypogastric and femoral regions, before and after 1 week's total fasting. In the femoral adipocytes the lipolytic sensitivity to isopropyl noradrenaline and the specific binding of the radioligands ^{3}H-dihydroalprenolol and ^{125}I-cyanopindolol decreased significantly, essentially owing to a reduction in the receptor density. In adipocytes from the hypogastric region no changes were found. The antilipolytic sensitivity to clonidine and the specific binding of ^{3}H-yohimbine decreased to the same extent in both regions during fasting. The findings suggest that the regulation of the lipid mobilization by the sympathetic nervous system during fasting occurs in part through changes in the adrenergic receptor density of the adipocytes. They also explain why mobilization of fat during fasting occurs more rapidly from the hypogastric than from the femoral regions.

134. Metabolism and transport of nutrients in the growth-retarded and malformed offspring of diabetic rats

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We have previously shown an incidence of 15–20% skeletal malformations and an increased teratogenic susceptibility during gestational days 2–7 in fetuses of diabetic rats. The fetal zinc content was markedly decreased, copper remained unchanged and manganese slightly increased in growth-retarded and malformed fetuses in late diabetic pregnancy. Treatment of pregnant diabetic rats with extra zinc in the drinking water (1, 15 and 75 mg/l, 15, 230 and 1150 μmol/l respectively) failed to decrease the frequency of skeletal malformations, whereas a zinc-deficient diet (zinc content 1.2 ppm) produced skeletal malformations in the offspring of non-diabetic rats. The transport of zinc, amino-isobutyric acid and methylglucose from mother to embryo and fetus was found to be disturbed in both early (gestational day 11) and late (gestational day 20) diabetic pregnancy. The present results suggest that diabetic teratogenesis in the rat may be related to disturbances in both transport and metabolism of nutrients in mother and conceptus.

135. Objective assessment of vibration perception at the thumb for early diagnosis and follow-up of sensory neuropathy

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Vibration perception threshold (VPT) was studied as a detection method for early polyneuritis. Risk factors for sensory neuropathy

were determined. VPT was measured three times sequentially on the thumb (T), the medial malleolus (M) and the big toe (B) in 226 volunteers and 120 Type 1 (insulin-dependent) diabetic patients with a Biothesiometer. Reproducibility (4 week interval) was tested in 20 subjects. In controls, VPT of M (2.19 ± 0.41, mean $\pm$ SD) was higher than that of T (1.43 ± 0.30) and B (1.98 ± 0.47). Multiple regression analysis, where age, sex, smoking and drinking habits were entered as independent variables, showed that VPT was positively correlated with age ($p < 0.005$), lower in females than in males ($p < 0.05$) and, on M, inversely correlated with alcohol use ($p < 0.05$). Smoking did not affect VPT. Intra-test and inter-test coefficients of variation were $\leqq 3\%$. In the 120 diabetic patients, VPT was more abnormal at T than at B and M ($p < 0.05$) and not different between sexes. In 60% of abnormal T VPT, polyneuritis had not been diagnosed before. Age at onset, but not duration of disease, was negatively correlated with VPT ($p < 0.05$). In conclusion, VPT, measured at the thumb, can be used as a method for early detection and follow-up of sensory neuropathy, and age at onset is a risk factor for polyneuritis.

136. Muscle metabolism in Type 2 (non-insulin-dependent) diabetes

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Peripheral hyperinsulinaemia, usually found in patients with diabetes, may have deleterious metabolic effects. Striated muscle cells were studied to examine this question. Biopsies from 12 Type 2 diabetic patients with significantly elevated fasting insulin (0.290 ± 0.053 nmol/l), blood glucose (8.3 ± 1.5 mmol/l), HbA_{1c} ($8.4 \pm 0.4\%$), fasting and 2 h post-prandial C-peptide (0.67 ± 0.18 and 2.41 ± 0.43 mmol/l) were compared with biopsies from 14 non-diabetic subjects undergoing minor surgery. The diabetic patients showed a marked triglyceride elevation in the striated muscle cells (290 ± 52 versus 48 ± 6.0 μmol/g; $p < 0.0005$). Moreover glucose-6-phosphate dehydrogenase (DH) (0.254 ± 0.03 versus 0.130 ± 0.01 U/g) and malic enzyme (0.15 ± 0.01 versus 0.05 ± 0.006 U/g), key enzymes in lipid synthesis, were significantly increased ($p < 0.0005$) in the hyperinsulinaemic diabetic patients, while glycolytic enzymes, hexokinase (0.65 ± 0.09 versus 1.82 ± 0.11 U/g), pyruvate kinase (7.3 ± 0.9 versus 13.2 ± 0.9 U/g), glyceraldehydephosphate DH (96 ± 9.9 versus 185 10.8 U/g) and α-glycerophosphate DH (7.3 ± 0.5 versus 12.5 ± 0.7 U/g) were decreased ($p < 0.001$ in all four cases). These data demonstrate substantial enhancement of lipid synthesis in skeletal muscle of diabetic patients with peripheral hyperinsulinaemia. In two non-diabetic animal models with peripheral hyperinsulinaemia, it has been shown that these changes are even more pronounced in the arterial wall. The altered substrate metabolism in skeletal muscle in diabetes may thus be associated with hyperinsulinaemia rather than with diabetes per se. If confirmed by further experiments, these findings have important implications with regard to macrovascular disease in diabetes.

137. Problem oriented participatory project work as a method of diabetes education in adults with Type 2 (non-insulin-dependent) diabetes

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At the Kisa Community Health Centre the therapeutic effects of a diabetes teaching programme (DTP) based on problem oriented participatory project work (POPPW) was assessed. Learner activity was studied in an interdisciplinary process together with educationalists. Recruited patients had to be older than 55 years of age, live in a home of their own and have Type 2 diabetes. The therapeutic effects of the teaching programme were assessed by study of: (1) alteration in diabetes-related knowledge and ability to cope with the disease; (2) alteration in metabolic profile as estimated by HbA_1, and change in dietary habits studied by a dietary history method assessed by a dietician. Finally the relationships between the two sets of variables were studied. Forty-five patients entered the study, 27 took part in the POPPW and the control group comprised 18 subjects given conventional teaching. Significant improvement of HbA_1 levels ($p < 0.05$) was found as well as an increase in knowledge in the project group after the intervention period. This was directly related to the POPPW as patients who completed the study did not alter therapy.

138. Interaction between insulin receptors and major histocompatibility complex antigens

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The possible relationship between insulin receptors and major histocompatibility complex (MHC) antigens was investigated. This study was initiated following the observation that Daudi cells, a human Burkitt lymphoma cell line, express neither Class I MHC antigens nor insulin receptors. Insulin receptors from $H\text{-}2^k$ mouse liver membranes were specifically labelled with an ^{125}I-photoreactive insulin analogue coupled to the α-subunit of the receptor by ultra-violet irradiation. When membranes were solubilized in 1% Nonidet P-40, five different monoclonal antibodies reacting with $H\text{-}2K^k$ precipitated up to 25% of the recoverable labelled insulin receptors, identified by SDS-polyacrylamide gel electrophoresis and autoradiography. No immunoprecipitation was observed with control mouse IgG or anti-$H\text{-}2K^b$ monoclonal antibodies. By contrast, insulin receptors labelled on $H\text{-}2^b$ mouse liver membranes could be precipitated only by anti-$H\text{-}2K^b$ monoclonal antibodies and not by anti-$H\text{-}2K^k$ monoclonal antibodies. Taken together these results suggest that Class I MHC antigens and insulin receptors form non-covalent complexes in mouse liver membranes. This relationship may represent a sufficient requirement for transmodulation of insulin receptors and MHC antigens which might be involved in the pathogenesis of certain forms of insulin resistance.

139. Occlusive arteriopathy of the lower limbs: prevalence of some vascular risk factors in diabetic and non-diabetic subjects

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To assess the prevalence of some vascular risk factors in the occlusive arteriopathy of the lower limbs of diabetic patients compared with normal subjects, 100 patients with arteriopathy (50 diabetic and 50 non-diabetic) of similar age were investigated. The stage of arteriopathy was more advanced in the diabetic patients. Male sex predominated in both groups, however significantly less in diabetics ($p < 0.01$). Obesity was more common in diabetes ($p < 0.001$). Arterial hypertension was also more evident in diabetes ($p < 0.01$), even in the absence of nephropathy. The role of smoking was less evident in diabetes ($p < 0.01$), possibly because of the presence of more women, rather than non-smokers. Sedentary life style was more often present in diabetes ($p < 0.01$). Total-, LDL-, and HDL-cholesterol, triglycerides, and total lipids did not differ ($p > 0.05$), neither globally, nor in both sexes taken separately. Serum uric acid and fibrinogen did not differ between the two groups ($p > 0.05$). The results show that the investigated risk factors are present both in diabetic and non-diabetic patients with occlusive arteriopathy of the lower limbs. However, the prevalence of some of them differs significantly.

140. Exercise induced changes in urinary albumin excretion rate and renal haemodynamics in Type 1 (insulin-dependent) diabetes

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It has been suggested that exercise can unmask a silent lesion by raising intra-glomerular pressure in patients with Type 1 diabetes who have otherwise normal renal function. To evaluate this concept we studied renal haemodynamics and urinary albumin excretion rate (UalbV) during exercise. Three age-matched groups were studied: 10 controls (C); 16 Type 1 diabetic patients with normal UalbV (D_1); and 14 Type 1 diabetic patients with microalbuminuria (15–150 μg/min, D_2). Group assignment was based on the mean of three 24 h outpatient urine collections. D_1 and D_2 were similar in mean duration of disease (13.3 and 15.5 years), HbA_{1c} (8.4%, 8.7%) and degree of other complications. The response of UalbV was similar in groups C and D_1 (basal→maximum, 5.5→8.3 versus 5.7→8.4 μg/min), but was exaggerated in group D_2 (49.7→119.4 μg/min). The qualitative changes in GFR and renal plasma flow (RPF) were similar in all three groups: GFR decreased by 12, 18, and 21%, and RPF decreased by 27, 35, and 36% in groups C, D_1, and D_2, respectively. The filtration fraction in both groups D_1 and D_2 (0.29) was significantly elevated above the level seen in group C (0.26) during exercise. These data suggest that exercise is not a sensitive tool for detecting early diabetic renal disease. Patients with normal UalbV do not demonstrate a significant increase in UalbV even when intra-glomerular pressure is raised further.

141. Insulin stimulates pyruvate kinase phosphatase activity in isolated rat hepatocytes

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Hepatic pyruvate kinase-L can be phosphorylated and inactived by a cyclic AMP-dependent protein kinase, and dephosphorylated and reactivated by protein phosphatase(s). Experiments carried out in isolated hepatocytes have shown that insulin accelerates the reactivation

of pyruvate kinase-L. However, there is no evidence that this effect is mediated by the stimulation of pyruvate kinase phosphatase activity. We have studied the influence of insulin on both pyruvate kinase and pyruvate kinase phosphatase activities in isolated rat hepatocytes. The phosphatase activity was assayed by measuring the reactivation of a partially purified glucagon-inactivated pyruvate kinase-L. Insulin (1500 mU/l) caused a transient reactivation of pyruvate kinase (30% over basal value) and simultaneously provoked a transient stimulation of pyruvate kinase phosphatase activity (40–60% over basal value). This insulin effect on phosphatase activity was dose-dependent, 3 mU/l being the calculated concentration corresponding to the half-maximal stimulation. Our results demonstrate that insulin exerts a short-term regulation on hepatic pyruvate kinase phosphatase activity. Studies to elucidate whether or not insulin provokes a stable modification of the phosphatase molecule are in progress.

142. Urinary C-peptide in 2-h urine collections after glucagon or breakfast: a valuable index of β-cell secretion in diabetes

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To determine how well 2-h urinary C-peptide (UCP) collections reflect the standard measures of β-cell function in a group of 16 subjects (six Type 1 (insulin-dependent) and six Type 2 (non-insulin-dependent) diabetic patients without nephropathy or autonomic neuropathy and four control subjects), we performed the following studies: (1) 24-h UCP collections; (2) 2-h UCP collections before and after intravenous glucagon (1 mg); (3) 2-h UCP collections before and after breakfast (400 kcal, 55% carbohydrates). Samples for plasma C-peptide (PCP) were obtained during the tests. PCP and UCP were estimated by radioimmunoassay. Two-hour baseline UCP (range 0.22–19.52 pmol/min) was correlated with baseline PCP ($r=0.78$, $p<0.001$). Two-hour UCP after glucagon (range 0.22–26.95 pmol/min) and breakfast (range 0.22–38.5 pmol/min) were correlated with PCP peak values ($r=0.79$ and $r=0.71$, $p<0.001$). 24-h UCP (range 0.38–26.95 pmol/min) correlated best with 2-h UCP values after breakfast ($r=0.81$, $p<0.001$). According to PCP responses, the subjects were classified in to three groups (normal, residual or no β-cell function). 2-h UCP values after breakfast allowed the best discrimination between them. We conclude that 2-h UCP collections reflect PCP values and may be an alternative non-invasive measure of β-cell function in diabetes.

143. Hyperglycaemia impairs first-phase insulin secretion in normal subjects

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We studied 24 normal men (aged 26 ± 2 years, weight $99\pm2\%$ ideal, mean $\pm$ SEM) using a two-step glucose clamp, to determine whether hyperglycaemia blunts glucose-mediated acute insulin responses. A calculated volume of glucose (40 g/dl) was infused to raise blood glucose to a new level within 10 min, and the rate of infusion of glucose (10 g/dl) adjusted to keep blood glucose constant for 50 min. Blood glucose was then raised again. In eight subjects, glucose was raised sequentially from basal values of 4.5 ± 0.1 to 5.6 ± 0.1, to 7.6 ± 0.2 mmol/l; in eight from 4.5 ± 0.1 to 7.9 ± 0.2, to 9.7 ± 0.2 mmol/l and in eight, from 4.5 ± 0.1 to 10.6 ± 0.3 to 19.7 ± 0.5 mmol/l. For the first step, the increase in insulin during the first 6 min of the step (ΔI) was linearly correlated with the corresponding increase in glucose (ΔG), $r=0.745$, $p<0.0001$. $\Delta I/\Delta G$ was therefore used as a measure of response. There was a significant decrease in insulin response during the second step (3.7 versus 7.3 mU/mmol, $p=0.0002$, paired t-test), particularly at higher glucose increments. Thus acute insulin response to glucose is markedly impaired in normal subjects after acute elevation of glucose to concentrations commonly found in non-insulin-dependent diabetic patients, who indeed show similar impairment of acute insulin secretion.

144. Effect of insulin on the distribution and disposition of glucose in man: a new kinetic analysis

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Distribution and exchange of glucose through body pools, and the effects of insulin on these processes, are still poorly described. ^{3}H-3-glucose was injected into six overnight-fasted volunteers, and the plasma tracer disappearance curve was frequently sampled for 150 min. A 1 mU $\cdot$ min$^{-1}\cdot$ kg^{-1} euglycaemic insulin clamp was then performed for 180 min (plasma insulin $\simeq$ 100 mU/l). After 90 min of clamp, another tracer bolus was given, and its time-activity curve followed for the remaining 90 min. Three exponential components were discernible in all tracer curves. Several candidate models were tested and, based on stringent statistical criteria, a three-compartment parallel model was chosen. The glucose initial distribution volume was 0.22 ± 0.02 mmol/kg (i.e., identical with plasma); the two other pools had similar size (0.51 ± 0.07 and 0.53 ± 0.05 mmol/kg) but the former, assimilated to the insulin-independent tissues, exchanged more rapidly ($1.09\pm0.01\cdot$ min^{-1}) with plasma than the latter (= insulin-dependent tissues, $0.12\pm0.01\cdot$ min^{-1}). Basal glucose turnover and total distribution volume were 11.9 ± 0.7 µmol $\cdot$ min$^{-1}\cdot$ kg^{-1}, and $26\pm1\%$ of body weight, respectively. Insulin caused no change in the initial or the insulin-independent pool, a doubling (to 1.06 ± 0.17 mmol/kg, $p<0.02$) of the insulin-dependent pool, and a fourfold increase in total glucose turnover (44.2 ± 4.7 µmol $\cdot$ min$^{-1}\cdot$ kg^{-1}, $p<0.001$); endogenous glucose output was abolished. Higher glucose turnover rates were associated with larger increments in the insulin-dependent pool ($r=0.92$, $p<0.01$). In addition to stimulating glucose disposition and inhibiting glucose release, insulin leads to accumulation of free, exchangeable (presumably intracellular) glucose in insulin-sensitive tissues of man.

145. A method to quantify glucose metabolism by individual tissues in anesthetized rats

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Glucose metabolism was quantified in muscles (soleus, extensor digitorum longus, epitrochlearis, diaphragm, heart), white adipose tissue (WAT) and brain of the rat by a (^{3}H)2-deoxyglucose (2DOG) method, modified as follow: tissue 2DOG-phosphate is measured chemically; the experimental protocol allows us to ignore the rate constants for 2DOG kinetics; correction factors for isotope effect are, except for the brain, determined in vitro; they differ for each tissue but are equal in presence or in absence of insulin (1 mU/ml). In the fed state (plasma insulin, 0.1 mU/ml), brain glucose utilization per unit weight is similar to that reported previously; heart and diaphragm use respectively ten- and fourfold more glucose than non-working muscles; WAT utilization is very low. During hyperinsulinaemic (4 mU/ml) euglycaemic clamp, brain and heart glucose utilization does not increase, but other muscles and WAT show a threefold increase, as for overall glucose turnover rate. After 48 h fasting (plasma insulin, 0.03 mU/ml), brain decreases glucose utilization by 30%, heart by 80%, other muscles and WAT by 50%, as for the glucose turnover rate. This method will be useful to quantify glucose utilization by individual tissues in various physiological states.

146. Response of ketone body metabolism to exercise during the transition from the post-absorptive to the fully fasted state

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Twenty-one normal subjects, fasted for 15 h to 5 days with ketone body (KB) levels ranging from 0.2 to 6.2 mmol/l, were exercised for 2 h on a treadmill at 50% of their $\dot{V}O_{2max}$. The kinetics of KB were measured before and during work using an infusion of ^{14}C-D(-)-β-hydroxybutyrate. After 2 h of work, the concentration and the rates of production and disposal of KB were increased by approximately 100% in the non-ketotic subjects, the rise in the metabolic clearance rate approximating 30%. These stimulatory effects became less and less marked as basal ketonaemia rose, the variables even being depressed by exercise when resting KB levels exceeded 5 mmol/l. The inhibition of the ketogenic response to work was unrelated to the plasma non-esterified fatty acid response and could be ascribed to the gradual inhibition of the hypoinsulinaemic effect of exercise that was associated with the rise in basal ketonaemia. The fact that the exercise-induced changes in production and uptake of KB were similarly affected by the degree of basal hyperketonaemia suggests that the regulation of hepatic and muscular metabolism of ketones is closely coordinated during work at all KB levels encountered during fasting.

147. Suppression of intestinal content by vasoactive intestinal polypeptide, somatostatin and gut glucagon-like immunoreactivity in long-term glibenclamide treatment in normal rats

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We reported previously that chronic sulphonylurea treatment depresses the response of pancreatic insular A, B and D cells to metabol-

ic stimuli and glucagon, insulin and somatostatin insular content. Since gastrointestinal hormones seem to affect insular secretion, we evaluated the effects of long-term sulphonylurea treatment on vasoactive intestinal polypeptide (VIP), somatostatin and intestinal glucagon-like immunoreactivity (gut-GLI) in 10 male Sprague Dawley rats treated daily with glibenclamide (1 mg/kg) for 4 months. Animals were killed 24 h after the last dose and the duodenum-jejunal VIP, somatostatin and gut-GLI contents were assayed and compared with measurements obtained from six control rats. Intestinal VIP, somatostatin and gut-GLI contents was depressed by sulphonylurea treatment (VIP: 786 ± 101 versus 1251 ± 289, $p < 0.1$; somatostatin: 33.3 ± 3.8 versus 94.9 ± 20.2, $p < 0.0025$; gut-GLI: 20.21 ± 7.14 versus 215.7 ± 81.1, $p < 0.05$). We concluded that long-term glibenclamide treatment clearly reduces intestinal VIP, somatostatin and gut-GLI contents. The impaired insulin response to metabolic stimuli in chronic sulphonylurea-treated rats may be due, at least in part, to insulinotropic gastrointestinal hormone inhibition by glibenclamide.

148. To extract or not to extract?: optimization of insulin antibody assays

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Using a polyethylene glycol (PEG)-based insulin antibody assay, we investigated the effects of (1) extraction of pre-bound insulin, (2) serum volume, (3) and incubation conditions and compared results with an immunoglobulin-specific (Ig) method in 24 sera. Sera were extracted with Dixon's method. Tubes contained either 10 or 30 µl unextracted or extracted sera. Conditions were 37 °C for 2 h ± an additional 4 °C overnight incubation, or 4 °C incubation alone. ^{125}I-antibody-bound insulin was precipitated with PEG. In addition, antibody-bound insulin in 10 µl of extracted sera were precipitated by antihuman Ig. Extraction increased non-specific binding by 0.1–1.2%. With non-specific binding subtracted, extraction increased percentage binding by a median of 9.4% ($p < 0.001$). There were no systematic differences between comparable assays containing 10 or 30 µl. 37 °C/2 h + 4 °C > 4 °C alone > 37 °C/2 h ($p < 0.001$). PEG and Ig-specific methods were correlated ($r > 0.90$, $p < 0.001$). Further, values did not differ significantly between PEG methods, using extracted sera, and optimal conditions and anti-Ig. Antibody assays using extracted sera provided additional sensitivity with results fully comparable to more expensive Ig-specific methods.

149. Abnormalities of left ventricular function in middle-aged Type 1 (insulin-dependent) diabetic patients: cardiomyopathy versus coronary heart disease

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Heart disease is the commonest cause of death in diabetic patients over 30 years of age. Using radionuclide ventriculography at rest and during cold-pressor stimulation, isometric exercise and supine dynamic exercise on a bicycle ergometer, we compared 22 asymptomatic insulin-dependent patients (aged 35–53 years, mean 43.0 ± 1.0 years); duration 7–23 years (mean 18.1 ± 0.9 years) with 11 healthy control subjects. All had normal resting ECG. Resting left ventricular ejection fraction (LVEF) was $46.8 \pm 1.5\%$ and $46.0 \pm 1.5\%$ in diabetic and control subjects respectively (NS), and fell by $0.5 \pm 1.2\%$ and $2.7 \pm 2.0\%$ in each group (NS) during isometric exercise, and by 0.2 ± 0.9 and 1.0 ± 1.3 (NS) during cold-pressor testing. During dynamic exercise LVEF rose by $9.2 \pm 1.5\%$ in diabetics and $14.8 \pm 2.2\%$ in controls ($p < 0.05$). Five diabetic patients showed an abnormal response to dynamic exercise, two of whom had an abnormal exercise ECG indicating coronary heart disease. A further three patients had an abnormal exercise ECG with a normal LVEF response to dynamic exercise. Thus, abnormalities on exercise testing suggesting coronary heart disease are common in asymptomatic middle-aged diabetic patients. In three patients the cause of abnormal LVEF is unexplained, but may be caused by a specific cardiomyopathy.

150. Defective stimulus-secretion coupling of human insulinoma is associated with disturbances in the regulation of transmembrane Ca^{2+} fluxes

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Regulation of β-cell function was examined using pieces of a benign tumour removed from the head of the pancreas of a 60-year-old woman. Immunocytochemical staining confirmed the presence of insulin-containing cells in an abundant mature fibrous matrix with no demonstrable glucagon or somatostatin. After 3 days of culture in RPMI-1640 supplemented with glucose 11.1 mmol/l, tumour pieces (140 ± 8 µg, mean ± SEM) released 19–54 mg insulin/kg dry weight and 11–15 mg C-peptide/kg during acute 60-min incubations with the concomitant uptake of 2.0–2.7 mmol ^{45}Ca/kg into the intracellular lanthanum-non-displaceable pool. At Ca^{2+} (2.56 mmol/l), addition of glucose (5.6–16.7 mmol/l) alone or in combination with theophylline (5 mmol/l), K^+ (25 mmol/l), glyceraldehyde (10 mmol/l), mannoheptulose (15 mmol/l), diazoxide (0.54 mmol/l), verapamil (50 µmol/l), D-600 (20 µmol/l) or trifluoroperazine (25 µmol/l) failed to modify hormone release or ^{45}Ca uptake. A close correlation was obtained between insulin and C-peptide output (molar ratio 1.3 ± 0.1). Further prolongation of culture produced a decline in hormone release which was unaffected by diazoxide (0.54 mmol/l) or verapamil (50 µmol/l), and attained a low but steady output of 13–19 ng insulin/dish per 24 h and 7–14 ng C-peptide/dish per 24 h by 7–17 days. The in vitro properties of this unresponsive insulinoma are consistent with its atypical clinical characteristics, and appear to stem from disturbances in the regulation of transmembrane Ca^{2+} fluxes.

151. Insulin binding to stomach epithelium: presence of a very high affinity component

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Insulin receptors were investigated in (1) glands isolated from guinea-pig gastric epithelium; (2) a human gastric cancer cell line. The binding of porcine ^{125}I-insulin (0.07–0.09 nmol/l; 10.5–13.5 mU/ l) to gastric glands was time- and temperature-dependent. At apparent equilibrium (2 h at 15 °C) 50% inhibition of tracer binding was obtained with unlabelled insulin 0.2–0.5 nmol/l (30–75 mU/l), indicating the presence of an unusual high affinity component of binding. This was not due to a species difference of guinea-pig receptors since a similar property was also found in a cell line (HGT-1) established from a gastric cancer of human origin: competition curves at apparent equilibrium (3 h at 20 °C) showed a 50% inhibition of binding of tracer (0.09–0.13 nmol/l; 13.5–19.5 mU/l) with a maximum of 0.5 nmol/l (75 mU/l) unlabelled insulin. Insulin in vivo triggers various effects on stomach (stimulation of acid and gastrin secretions, of thymidine incorporation, and inhibition of somatostatin release), but whether these actions are direct or indirect (vagal stimulation) remains controversial. Our results showing the high sensitivity of the insulin binding sites suggest that gastric receptors could be occupied at low insulin concentrations occurring during the pre-absorptive phase of food intake.

152. The Islington diabetes survey: how useful is the glycosylated haemoglobin assay as a diagnostic test for diabetes?

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One thousand subjects, over the age of 40 years, randomly selected from a general practice population have been screened for diabetes by capillary blood glucose estimation 2 h after a 75 g glucose load using modified World Health Organisation diagnostic criteria. Glycosylated haemoglobin (HbA_1) was assayed on undialysed blood samples collected with 2-h blood glucose samples by an electroendosmotic method (Corning). 24 subjects (2%) were classified as having diabetes, 40 (4%) as having impaired glucose tolerance and 917 (92%) had normal tolerance. 22 (2%) were unclassifiable, no 2 h blood glucose value being obtained. Sensitivity, specificity and predictive value (positive test) for HbA_1 in diagnosing diabetes were calculated at different cut-off levels. At 8.5% the assay's sensitivity was 82.6%, specificity was 92.4% and its predictive value (positive test) was 22.6%. At 9.0%, sensitivity falls to 73.9%. Increasing the cut-off level to 10.5% improves the specificity to 99.4% and the predictive value to 76.9% but the sensitivity falls to 43.5%. While 8.5% may prove a useful upper normal limit for the assessment of metabolic control in diabetes, higher cut-off levels may be necessary when using the HbA_1 assay as a diagnostic screening test.

153. The miscibility of human soluble and ultralente insulin: a study in normal and diabetic subjects

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Many diabetic patients mix insulins in the syringe prior to injection. We have studied the effect of mixing human neutral soluble insulin (Actrapid, 100 U/ml) with human ultralente insulin (crystalline zinc

suspension, Ultratard, U 100 U/ml). Eight normal subjects and six C-peptide-negative diabetic patients received 6 U (diabetics 9 U) human Actrapid and 14 U (diabetics 21 U) human Ultratard insulin either drawn up and injected separately or mixed together for 60 s in the syringe, in random order. Injections were given at 0900 h into the anterior abdominal wall. Normal subjects fasted. Diabetic patients ate their normal breakfast and lunch. Frequent blood samples were taken for measurement of blood glucose, serum insulin and C-peptide (normal subjects) or plasma free insulin (diabetic). In normal subjects blood glucose fell more rapidly ($p < 0.01$ at 45 min) and serum insulin (corrected for endogenous insulin secretion) rose more rapidly ($p < 0.05$ at 15, 30, 45, 115, 120 min) reaching a greater peak level (17.2 ± 1.8 versus 14.2 ± 1.9 mU/l at 75 min) when the insulins were injected separately. Similarly in diabetic patients the rise in blood glucose following breakfast was less ($p < 0.05$ at 15, 30, 45 min) and rise in free insulin more rapid ($p < 0.05$ at 15, 30, 45, 105 min) reaching a greater peak level (27.9 ± 6.8 versus 21.3 ± 2.1 at 150 min) with separate injections. Mixing with human Ultratard in the syringe significantly blunts the onset of action of human Actrapid insulin.

154. Preparation and characterization of split forms of human proinsulin

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Split forms of human proinsulin (HPI) are present in the pancreas and sera of normal individuals. Using HPI isolated from recombinant DNA sources, the split forms of HPI were prepared using enzymatic cleavage procedures and isolation by preparative high performance liquid chromatography. Receptor binding of each derivative was evaluated using IM-9 lymphocytes, isolated rat adipocytes and partially purified rat liver plasma membranes. Biological potency was evaluated using stimulated glucose incorporation into total lipids in isolated adipocytes and blood glucose lowering in rats. The derivatives split between the connecting peptide and A-chain exhibited approximately 20-fold increases in activity compared with HPI. These increases were similar to those for analogous animal proinsulin intermediates. Surprisingly the derivatives split between the B-chain and connecting peptide exhibited an approximate fivefold increase in receptor binding and biological activity representing a significantly greater increase than has been reported for analogous animal proinsulin derivatives. The availability of these derivatives is important for evaluating the biological activity and potential metabolism of HPI. In addition, their availability as standards allows clearer definition of the specificity of HPI antisera.

155. Does maternal glucose homeostasis really deteriorate in pregnancy?

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Studies using oral glucose tolerance tests and liquid formula test meals suggest a deterioration of glucose homeostasis in late pregnancy. This effect occurs despite the universal finding of lower fasting plasma glucose in pregnancy, and higher basal and post-prandial plasma insulin levels. It has been suggested that pregnancy is associated with peripheral insulin resistance. We have studied two groups of normal weight non-diabetic European women: group A – non-pregnant ($n = 15$), group B – pregnant 32–36 weeks ($n = 14$). They ate identical diets (1,900 Kcal, 40% carbohydrate, 40% fat, 10 g dietary fibre). Hourly plasma samples were obtained between 09.00 and 19.00 h. Plasma glucose and insulin levels were compared by the Wilcoxson rank sum test. The post-prandial glucose peaks were higher after meals in group A. The only time the glucose levels of group B were higher than A was in the second hour after breakfast and at the evening meal – at all other time points the glucose levels in group B were significantly lower than those in group A. There was a trend to higher insulin levels in group B, but the differences were rarely significant. We suggest that late pregnancy is a state of *improved* glucose homeostasis.

156. Plasma retinol and diabetes mellitus

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An inverse relation between plasma retinol levels and cancer incidence has been found in non-diabetic subjects and some studies have shown a decreased cancer risk amongst diabetic patients, while others have not. Plasma retinol levels have therefore been compared between groups of middle-aged diabetic and non-diabetic control subjects. They were measured in duplicate by high performance liquid chromatography in 65 Caucasoid diabetic patients (39 males; 26 females) in the age range 35–64 years, and 157 Caucasoid control subjects (109 males; 48 females) in the same age range recruited from the Northwick Park heart study. For Type 2 (non-insulin-dependent) diabetic patients, mean plasma retinol levels were higher than those of controls (males 663 versus 606 μg/l, NS; females 619 versus 532 μg/l, $p < 0.05$). However, for Type 1 (insulin-dependent) diabetic patients, mean plasma retinol concentrations were significantly lower than control levels (males 494 versus 606 μg/l, $p < 0.01$; females 455 versus 532 μg/l, $p < 0.05$). The nutritional and metabolic determinants of plasma retinol may therefore differ between the main types of diabetes. For all diabetic patients combined, those with cardiovascular disease, proteinuria, or retinopathy (background or proliferative) had significantly higher retinol levels compared with those without complications (578 versus 480 μg/l, $p < 0.01$).

157. Effect of bepridil on insulin secretion

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Bepridil, a new anti-anginal drug, appears to act, in part, through an inhibition of slow inward Ca^{2+} current. Glucose-induced insulin secretion depends on extracellular calcium and various 'calcium antagonists' inhibit this secretion. The effects of bepridil on insulin release were studied. The drug was compared with verapamil, using rat isolated islets incubated in vitro. Glucose-induced stimulation (8 or 16.7 mmol/l) of insulin secretion was inhibited in a concentration-dependent manner by either bepridil (0.5–10 μmol/l) or verapamil (5–40 μmol/l). For example, insulin secretion in control islets (16.7 mmol/l glucose) was 11.09 ± 0.31 ng·islet^{-1}·h^{-1}, whereas in the presence of bepridil (10 μmol/l) secretion was completely inhibited (0.7 ng·islet^{-1}·h^{-1}, $p < 0.001$). Verapamil was approximately ten times less potent than bepridil. High concentrations of bepridil (35–70 μmol/l) were less inhibitory than lower concentrations. This may be related to the stimulation of basal insulin secretion by the highest concentration of bepridil (control, 1.89 ± 0.51 ng·islet^{-1}·h^{-1}; bepridil (70 μmol/l), 3.88 ± 0.62 ng·islet^{-1}·h^{-1}, $p < 0.05$). Pretreatment of anaesthetized rats with bepridil (5 mg/kg intravenously) did not modify glucose-induced elevations of plasma insulin. The discrepancy between the findings in vivo and in vitro remains to be explained. Studies in man have not revealed any deleterious effect of bepridil on glucose tolerance or insulin secretion.

158. Mitogen stimulation of fractionated $T3^+$, $T4^+$ and $T8^+$ cells in Type 1 (insulin-dependent) diabetes

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Although conflicting results on T cell subpopulations in Type 1 diabetes have been reported, suppressor cell dysfunction or deficiency is held responsible for the production of autoantibodies in Type 1 diabetes. To evaluate the function of each lymphocyte subset, we studied blastogenic response of lymphocytes fractionated into individual $T3^+$, $T4^+$, $Ia1^+$ and $T8^+$ subsets in 10 newly discovered Type 1 diabetic patients (mean age 13.2 ± 6.3 years). To separate T cell subjects we used anti-mouse-IgG-coated plates incubated with anti-Ia1, -T3, -T4 and -T8 monoclonal antibodies and lymphocyte suspension (5×10^6 cells). Cells from the supernatants were $T3^+$, $Ia1^+$, $T8^+$ and $T4^+$ subsets, respectively. Total cells, single subsets and co-cultured individual subset recombinations (2×10^6 cells) were stimulated with phytohaemagglutinin (PHA) (1 μg/ml) or concanavalin A (10 μg/ml) and cultured for 3 days at 37 °C in 5% CO_2 atmosphere. The proliferative responses were measured by ^{3}H-thymidine incorporation. The mean ± SD indices of blastogenesis to PHA and concanavalin A respectively were the following: total cells: 60 ± 17.4 and 22.3 ± 10.8; $T3^+$; 17.3 ± 7.2 and 2.8 ± 1.1; $T4^+$; 7.9 ± 4.7 and 2.8 ± 1.6; $T8^+$, 20.5 ± 8.7 and 4.5 ± 3. Moreover, when individual subsets of $T4^+$ (1×10^6 cells) and $T8^+$ (1×10^6 cells) were recombined, the blastogenic response for PHA was 21.5 ± 4.2 and for concanavalin A 6.1 ± 3.5, hence no different from single $T8^+$ blastogenesis. These results suggest that $T8^+$ cells in Type 1 diabetes have a normal blastogenic response and that the reported suppressor-cell activity deficit is not due to the functional deficit of $T8^+$ cells but probably to their decreased number or to imbalance in immunoregulatory interactions in the early phases of Type 1 diabetes.

159. Insulin receptors in rat brain cortex: subunit structure and autophosphorylation

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Insulin receptors are widespread in rat brain and our studies have shown binding characteristics different from other tissues: distinct peptide specificity and absence of negative cooperativity. In all other tissues studied so far insulin receptors consist of α-subunits (M_r 130,000) which bind insulin, and β-subunits (M_r 95,000) whose phosphorylation is stimulated by insulin. To evaluate whether receptors in brain show similar composition, their structural and functional characteristics were studied. Purified synaptosomes were affinity-labelled with photoreactive ^{125}I-insulin, solubilized, and incubated with anti-insulin, anti-receptor or normal serum. After precipitation with protein-A, SDS-polyacrylamide gel electrophoresis revealed one radioactive protein (M_r 130,000) precipitated with anti-insulin or anti-receptor serum, but not with normal serum. This indicates the presence of insulin-receptor α-subunits. Solubilized synaptosomes were phosphorylated with (γ-^{32}P)ATP in absence or presence of insulin. SDS-polyacrylamide gel electrophoresis of immunoprecipitates obtained with normal serum showed few labelled proteins. Only one additional phosphoprotein (M_r 95,000) was seen after immunoprecipitation with anti-receptor serum and its phosphorylation was increased by insulin. This shows the presence of insulin-receptor β-subunits. In conclusion, insulin receptors in rat brain cortex consists of α- and β-subunits with separate functions: insulin binding and insulin-stimulated autophosphorylation.

160. Development of scales to measure perceived control of diabetes mellitus and diabetes-related health beliefs

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A series of scales was designed to measure perceived control of diabetes and diabetes-related health beliefs with a view to predicting treatment preferences and individual differences in response to the treatments. Scale development is described. The psychometric properties of these scales were examined with responses from 286 insulin-requiring adult diabetic patients. The newly developed scales showed that these patients were significantly more likely to attribute responsibility for their diabetes control to themselves rather than to their medical advisers or to other factors ($p < 0.001$). For most of the patients the benefits of treatment were perceived substantially to outweigh any barriers ($p < 0.001$). Compared with their perceptions of vulnerability to disorders unrelated to diabetes, patients perceived themselves to be more vulnerable to diabetes-related complications, such as eye, kidney and foot problems ($p < 0.001$) but not to heart disease.

161. Calcium-independent stimulation of adenylate cyclase and insulin release in pancreatic islets by forskolin

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Forskolin (0.3–30.0 μmol/l) caused a dose-related activation of adenylate cyclase in a particulate subcellular fraction derived from rat pancreatic islets. Forskolin (5.0 μmol/l) increased the cyclic AMP content of intact islets six-fold whether in the absence or presence of D-glucose (2.8–16.7 mmol/l). Forskolin failed to affect D-[U-^{14}C]glucose oxidation, glucose-stimulated ^{45}Ca net uptake and basal insulin release, but markedly enhanced insulin output in the presence of glucose (5.6–27.8 mmol/l). As little as forskolin 0.15 μmol/l was sufficient to cause a significant increment in insulin output, the maximal response being recorded at forskolin concentrations in the 1.5–15.0 μmol/l range. Forskolin also augmented insulin release evoked by 2-ketoisocaproate, L-leucine (alone or in combination with L-glutamine), 12-*o*-tetradecanoylphorbol-13-acetate, or Ba^{2+} (alone or in combination with theophylline). Forskolin stimulated insulin release and cyclic AMP content in Ca^{2+}-deprived but glucose-stimulated islets. These data indicate that (1) an increase in the cyclic AMP content of the islets is unable per se to cause insulin release, (2) the enhancing action of endogenous cyclic AMP upon insulin release evoked by either nutrient or non-nutrient secretagogues does not result from an increased net uptake of calcium, and (3) such an enhancing action persists in the absence of extracellular Ca^{2+}.

162. Humoral immunity in diabetic pregnancy: insulin-anti-insulin complexes and insulin antibodies

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Insulin antibodies (IAb) and insulin-anti-insulin complexes (Ins/iAb) were investigated in 23 diabetic women during pregnancy and in their infants. All the patients were sampled at the beginning and at the end of pregnancy, and all but two were treated with highly purified insulins during pregnancy: 13 had Type 1 (insulin-dependent), seven Type 2 (non-insulin-dependent) and three gestational diabetes. IAb were found at the end of pregnancy in 6 out of 13 of the Type 1, 6 out of 7 of the Type 2 and 2 out of 3 of the gestational diabetic patients. These antibodies were also present in 11 out of 23 of the infants studied. Ins/iAb were found at the beginning of pregnancy in 38% of the mothers with Type 1 diabetes and in 29% of those with Type 2, and at the end of pregnancy in 15% and 29% respectively. Ins/iAb were also found in 38% and 42% of newborn infants of Type 1 and Type 2 diabetic women respectively and were more strongly correlated with the occurrence of Ins/iAb in their mothers at the beginning than at the end of pregnancy. Thus, in contrast to IAb, Ins/iAb present in the neonatal circulation do not parallel those in the mothers at the end of pregnancy. Their pathophysiological consequences are considered.

163. Secretin elevates the 10000- and 4200-dalton immunoreactive human pancreatic polypeptide plasma components in normal subjects and patients with multiple endocrine adenomatosis Type 1

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Although secretin increases plasma immunoreactive human pancreatic polypeptide (IRHPP), its action upon the different plasma IRHPP-components is not known. A secretin (KabiVitrum, Stockholm) "bolus" (2 U/kg) was intravenously injected into seven normal subjects and five multiple endocrine adenomatosis Type 1 (MEA-1) patients. Plasma samples were obtained, and basal and post-secretin IRHPP-peak filtered on Biogel P-30 (normal = 5, MEA-1 = 2). Samples were assayed using Dr. Chance's No 615-antibody. Basal plasma IRHPP was 77 ± 13 pg/ml (Mean ± SEM) in normal subjects and 1468 ± 695 pg/ml in MEA-1 patients, rising at 5 min to 325 ± 93 ($p < 0.01$) and 4253 ± 1275 pg/ml ($p < 0.05$), the maximal increments ranging from 14–639 and 1038–6240 pg/ml, respectively. The > 20000-, 10000-, 4200- and 2000-dalton IRHPP-components were present. Their corresponding basal levels in normal subjects were 47 ± 14, 11 ± 3, 33 ± 12, 5 ± 1 pg/ml; after secretin, only IRHPP^{10000} (101 ± 46 pg/ml $p < 0.05$) and IRHPP^{4200} (270 ± 91 pg/ml $p < 0.05$) were increased. In MEA-1 patients, basal values of the three smaller IRHPP-components were higher than the respective mean ± 2SD value in normal subjects; after secretin the IRHPP^{4200} accounted for 88–99% of the total IRHPP increment; the remainder was due mainly to IRHPP^{10000}. In conclusion, intravenous secretin increased plasma IRHPP-components of 10000- and 4200-dalton; in MEA-1 patients, there was a preferential rise of IRHPP^{4200} and both components contributed to their high basal plasma IRHPP. These findings provide additional evidence that IRHPP^{10000}, the putative pancreatic polypeptide precursor, is a co-secretory product of the pancreatic polypeptide cell.

164. Screening for diabetic nephropathy: which urine sample?

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A screening programme has been in progress to identify those diabetic patients at risk of developing nephropathy: those whose albumin excretion rate (AER) is > 30 μg/min. A timed overnight urine specimen was requested from each patient but the response rate was only 70%. Consequently, in the latter half of the study a midstream urine specimen was also taken for random urinary albumin concentration. Microalbuminuria levels were measured using a micro-ELISA technique. In 58 patients, where both types of sample were available, an assessment of the value of a screening test using a random urinary albumin concentration > 25 μg/ml to predict AER > 30 μg/min was made. The sensitivity of the test was 100%, specificity 71% but predictive value only 16%. In 175 patients timed overnight urine samples were available and the albumin concentration in the specimens was reviewed to assess the value of a screening test using the overnight concentration > 20 μg/ml to predict an AER > 30 μg/min. Here the sensitivity was 86%, specificity 97% and predictive value 71%. It is suggested that the albumin concentration of a urine sample collected

on rising would provide a suitable screening test to identify those diabetic patients at risk of developing nephropathy.

165. Effects of restoration of normal basal insulin levels on glucoregulatory responses to basal and 5 × basal adrenaline infusion in adrenalectomized dogs

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The adrenalectomized dog is euglycaemic, with normal glucose kinetics in the presence of hypoinsulinaemia and hyperglucagonaemia. As opposed to normal dogs, insulin (IRI) levels do not rise in response to adrenaline 0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$, a partial stress model. Insulin and glucose were infused for 2 h in four adrenalectomized dogs to re-establish normal circulating IRI levels and euglycaemia, then continued with concomitant adrenaline infusion (0.02 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 90 min followed by 0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 90-min). Intraportal insulin infusion (0.125 $mU \cdot kg^{-1} \cdot min^{-}$) increased circulating IRI levels (7.7 ± 0.8 to 13.3 ± 2.8 mU/l, $p < 0.05$). Glucose production (Ra) decreased (3.9 ± 0.2 to 2.5 ± 0.3 $mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.02$), with no change in glucose utilization. Plasma glucagon decreased (217 ± 24 to 182 ± 22 pg/ml, $p < 0.01$). Adrenaline (0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) caused a transient increase in IRI ($\Delta 21 \pm 6$ mU/l, $p < 0.05$), no increase in Ra and decreased glucose utilization (4.3 ± 0.2 to 3.0 ± 0.2 $ml \cdot kg^{-1} \cdot min^{-1}$, $p < 0.05$). In conclusion, (1) after adrenalectomy euglycaemia is maintained, as the 40% decrease in IRI prevents a 35% drop in Ra; (2) hypoinsulinaemia cannot account for > 30% of the observed hyperglucagonaemia; (3) normal sensitivity of β cells to high levels of adrenaline occurs only when normal basal insulin is restored; (4) the transient IRI rise prevents the rapid increase in Ra observed in adrenalectomized and normal dogs, thus hyperglycaemia results only from decreased glucose utilization. Thus, hypoinsulinaemia after adrenalectomy represents an important compensatory mechanism.

166. Approaches to the isolation of insulin-resistant hepatoma cells

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We have constructed a chimeric toxin in which pokeweed antiviral protein (PAP), a ribosomal inhibitory protein of plant origin, is conjugated to insulin via a disulphide linkage. PAP itself lacks a binding subunit and is not toxic to intact cells. Intoxication requires internalization of toxin into the cytoplasm; thus, mutants altered in insulin binding, internalization, or receptor-ligand processing are potentially obtainable by selecting cells resistant to the chimeric toxin. The free amino groups on the A1-alanine and B29-lysine of insulin, which are necessary for insulin binding, are first blocked with 2-(t-butoxycarbonyloximino)-2-phenyl-acetonitrile and the insulin derivative purified on SP-Sephadex. A disulphide linker is added to the free amino group on B1-phenylalanine using N-hydroxysuccinimidyl-3-(2-dithiopyridyl)-propionate (SPDP) and the insulin deblocked. PAP, purified from leaves or seeds of *Phytolacca americana,* is modified with SPDP and the chimeric toxin is formed by disulphide exchange. PAP-insulin (80 nmol/l) inhibited protein synthesis in hepatoma cells by 50%. This effect was blocked by a ten-fold excess of insulin, suggesting insulin-receptor-mediated cytotoxicity.

167. Plasma proteins in the urine from Type 1 (insulin-dependent) diabetic patients in different stages of nephropathy

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Urine from Type 1 diabetic patients was tested by crossed immunoelectrophoresis for proteins reacting with an antiserum against human serum proteins. This method permits the simultaneous determination of many serum proteins including unidentified proteins reacting with the antiserum, giving a more comprehensive determination than many specific immunochemical assays because it uses both immunochemical reactivity and electrophoretic mobility in the determination of the protein. The study included patients with onset of diabetes before age 40 years, duration of diabetes more than 5 years, and absence of bacteriuria and non-diabetic renal disease. The patients were divided into four groups of 10: patients with normal urinary albumin excretion, patients with microalbuminuria, patients with clinical diabetic nephropathy and normal serum creatinine, and patients with clinical nephropathy and elevated serum creatinine. The number of detectable proteins increased with the severity of clinical nephropathy. Up to 18 different serum proteins were found in urine from patients with severe clinical nephropathy. The increase in urinary albumin excretion exceeded that of IgG excretion in patients with microalbuminuria when compared with patients having normal urinary albumin excretion. It is concluded that plasma protein excretion is selective in patients with microalbuminuria.

168. Regulation of insulin receptor from human erythrocytes in vitro

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Mature erythrocytes (RBC) are extensively used to study insulin receptor binding in different clinical conditions. However it is not proved that RBC insulin receptors (IR) present the same characteristics and behaviour as those of target cells of insulin action. In this study we have characterized in vitro: (1) the ability of a pre-incubation with insulin (from 10^{-9} to 10^{-6} mol/l, for 3 h at 37 °C) to down-regulate IR in erythrocytes; (2) the effect on insulin binding of the reducing agent DTT (from 0.1 to 10 mmol/l); (3) The P^{32} incorporation of purified insulin receptor from RBC. Our results show: (1) a 10% reduction of IR binding after insulin pre-incubation, at any tested concentrations. However IR complex internalization is undetectable at 37 °C; (2) a DTT-induced dose-dependent reduction of IR affinity; (3) an insulin stimulation of P^{32} incorporation in the β subunit (M_r 95,000) of the IR. Therefore IR of human RBC seem to present some structural and functional properties (response to DTT, autophosphorylation) similar to those of target cells of insulin action, but they are insensitive to a major physiological regulator such as insulin concentration. Consequently RBC should be used with caution to study insulin-receptor interaction for clinical purposes.

169. Glomerular basal membrane selectivity towards albumin in functional and clinical phases of diabetic nephropathy: studies on albumin conformation

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Conformational determinants for glomerular sieving of macromolecules have been shown to be important in rats. We have investigated the influence of the three-dimensional structure of albumin in determining its renal handling in 10 normal subjects and 24 diabetic patients (8 with normal albuminuria (group A), 8 with functional nephropathy (group B) and 8 with clinical nephropathy (group C), taking the free sulphydryl group (SH) content of albumin as a marker of its three dimensional structure. Albumin was purified with pseudoligand chromatography on Affi-Gel Blue and SH determined by the Elman procedure. Results are given as mol-SH/mol albumin. While the-SH concentration of serum albumin was 0.425 ± 0.002 in all patients, without any difference among groups, the urinary concentration was increased (compared with serum) in normal subjects 1.67 ± 0.54, (group A) 2.03 ± 0.52 and (group B) 2.01 ± 0.62 mol SH/mol albumin, giving a urinary/serum ratio respectively of 2.95 ± 0.54 (normal subjects), 4.65 ± 1.17 (group A) and 4.09 ± 1 (group B). Group C patients showed an SH concentration for urinary albumin of 0.37 ± 0.002 with a urinary/serum ratio of 0.66 ± 0.1 ($p < 0.001$ compared with other groups). These data provide direct evidence for the influence of the conformation of albumin in determining its renal handling in vivo. They further describe the difference between functional and clinical nephropathy in terms of renal selectivity towards albumin.

170. Glucagon-like peptide-1 has no glucagon-like effect on plasma glucose and insulin

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Glucagon-like peptide-1 (GLP-1) has the appearance of a highly conserved regulatory peptide encoded by mammalian glucagon mRNA, and shows an amino-acid sequence homology with glucagon of 14 out of 29 comparable residues. GLP-1-like immunoreactivity is found in the mammalian pancreas and may be co-secreted with glucagon. We have tested synthetic GLP-1-(1-37) at high dosage to look for glucagon-like effects on plasma glucose and insulin concentrations. Subcutaneous doses of 400 µg and 100 µg of GLP-1 were administered to 12 cortisone-pre-treated, fasted rabbits and compared with 24 µg and 6 µg doses of glucagon and diluent controls by a modification of the standard twin cross-over bioassay for glucagon. Plasma glucose concentrations 20 min after injection of GLP-1 (5.55 ± 0.27 mmol/l, mean ± SEM, 400 µg GLP-1; 5.47 ± 0.24 mmol/l, 100 µg GLP-1) were not significantly different from those after diluent injection (5.45 ± 0.16 and 5.77 ± 0.29 mmol/l) and corresponding immunoreactive insulin concentrations (8.1 ± 2.6 and 7.4 ± 2.5 mU/l) were slightly but

not significantly raised (diluent controls 5.3 ± 0.8 and 5.1 ± 1.0 mU/l). Glucagon produced the expected increases in plasma glucose and insulin concentrations. Thus, although GLP-1 is co-synthesised with glucagon, it has no direct glucagon-like effect on plasma glucose and insulin. Other possible intra- or extra-pancreatic roles remain to be discovered.

171. Brittle diabetes – all in the mind?

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Results of psychiatric and psychological examinations are reported in 19 young women with brittle diabetes (mean age 18.3 years, range 13–27 years), whose lives were disrupted by frequent ketoacidosis (mean 22 episodes/patient). All had extensive interviews with diabetologists exploring attitudes and behaviour of patient and family. Eleven had formal assessments by a psychologist, and eight by a psychiatrist. No abnormalities were found by the psychiatrist. Psychological testing showed a stereotyped pattern of group dependency and low self-sufficiency. The intensive interviews with experienced diabetologists revealed important problems. Fourteen exhibited a serenity and optimism at odds with their predicament, 10 had disturbed family relationships and 10 admitted to episodes of serious manipulation of therapy, which included stopping injections (5), massive over-eating (4), damage to infusion equipment (4), dilution of insulin infusions with tap water (2), and factitious hypoglycaemia (1). Extended interviews and observations by skilled diabetologists provided the information of diagnostic relevance. Referral to psychiatrists working in isolation was unrewarding, and in some cases misleading. Though metabolic abnormalities are documented in this group of patient, factitious instability is also present in about 50%, and may be of importance in the maintenance, and possibly the genesis of the syndrome of brittle diabetes.

172. Immunotherapy in the early phases of Type I (insulin-dependent) diabetes: immunological and metabolic studies

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Immunotherapy in Type I diabetes is suggested by the present knowledge that immunological mechanisms are involved in β-cell destruction. In animals, it has been demonstrated that methisoprinol prevents and cures hyperglycaemia in streptozotocin-induced diabetes. In 20 newly diagnosed Type I diabetic patients (15–60 days), we studied the influence of methisoprinol therapy (4 g/day for 3 months) both on immunological (immunoglobulins, complement fractions, autoantibodies, antiviral and islet cell antibody titres, T lymphocyte subsets) and metabolic parameters (daily insulin requirement, serum and 24/h urinary C-peptide). As controls, we examined 15 age- and diabetes onset-matched patients. Methisoprinol does not enhance organ-specific autoantibody production, even if it seems to favour the disappearance rate of islet cell antibodies. Both groups showed a reduction in $T8^+$ cells at the beginning of the study, but by the third month, while treated patients showed an increase on average (24.3 ± 6%), the untreated group had a further reduction (12.3 ± 1.9%, $p < 0.001$). By the third month, the treated patients showed a significant decrease in daily insulin requirement (13 ± 4.9 versus 22 ± 10 IU/day); five patients maintained satisfactory residual β-cell function and four had a honey-moon period (insulin-requirement < 0.5 IU/kg). Our data suggest that methisoprinol is not a polyclonal activator, does not seem to enhance autoantibody production, or to reduce immune-surveillance mechanisms, but positively intervenes in the preservation of β-cell function.

173. Exaggerated ketonaemic response to adrenaline infusion in brittle diabetic patients maintained at physiological plasma insulin levels

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An exaggerated rise in blood glucose levels in response to stress hormone infusion has been reported in diabetes of juvenile onset. To investigate whether liver ketogenesis is also abnormally sensitive to catecholamines, we infused adrenaline (1.2 μg/min per m^2 for 150 min) in four brittle diabetic patients (aged 30 ± 5 years; overweight +4 ± 2%) and in three control subjects of similar age. To avoid the influence of lack of insulin, it was infused in diabetic subjects (3.75 mU/min per m^2) until 5.6 mmol/l blood glucose was obtained and then the adrenaline infusion was superimposed for further 150 min. Blood samples were withdrawn every 30 min for adrenaline, IRI, glucagon, glycerol, non-esterified falty acid (NEFA), hydroxbutyrate (β-OH) and acetoacetate (AcAc) measurements. Although ketone body levels at 0 min (corresponding to glucose 5.6 mmol/l) were roughly similar in control (233 ± 11 μmol/l) and diabetic subjects (260 ± 77 μmol/l), the adrenaline infusion elicited an exaggerated increase in ketone body concentration (1320 ± 280 versus 350 ± 70 μmol/l in control subjects) in spite of the fact that their IRI levels had been clamped at control values (9.6 ± 1.0 mU/l). Likewise a higher rise in blood glucose levels was found in diabetic subjects (10.5 ± 1.1 versus 6.7 ± 0.5 mmol/l). Since during adrenaline infusion mean plasma NEFA (1191 ± 300 versus 1121 ± 218 μmol/l), glucagon (88 ± 19 versus 97 ± 22 pg/ml) and adrenaline (250 ± 14 versus 240 ± 20 pg/ml) values were similar in diabetic and control subjects, the exaggerated ketonaemic response of diabetic patient may be attributable to an inadequate ketogenic liver responsiveness to adrenaline.

174. Physiological inhibition of hexokinase in intact islets

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At a physiological glucose concentration (8.3 mmol/l), glucose phosphorylation by islet homogenates is attributable mainly to hexokinase, the relative contribution of glucokinase not exceeding 10–20%. In intact islets, however, glucose utilization at the same sugar concentration is three times higher than that observed at a low glucose concentration (1.7 mmol/l) sufficient to saturate hexokinase. We have sought an explanation for this disparity between enzymatic and metabolic data. Hexokinase activity was judged from the conversion of D(U-^{14}C)glucose to its phosphorylated products which were separated by anion-exchange chromatography. Hexokinase (V_{max} = 1.5 pmol/min per islet, K_m = 0.05 mmol/l) was present in both islets and purified β cells, and was inhibited non-competitively by glucose-6-phosphate (K_i: 0.13 mmol/l). Glucose-1,6-biphosphate also inhibited hexokinase (K_i: 0.2 mmol/l), whether in the absence or presence of glucose-6-phosphate. In intact islets, glucose (0–27.8 mmol/l) caused a sigmoidal increase in both glucose-6-phosphate (0.26–0.79 pmol/islet) and glucose-1,6-bisphosphate (5.1–47.7 fmol/islet) content. From these data and the intracellular volume, the rate of glucose phosphorylation as catalyzed by hexokinase ranged, in intact islets, from 26.2% (at glucose 2.8 mmol/l) to 12.9% (at glucose 27.8 mmol/l) of its value in islet homogenates. Thus, hexokinase is indeed severely inhibited in intact islets exposed to glucose.

175. Acute hyperglycaemia increases platelet sensitivity to prostacyclin in non-diabetic subjects

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Altered platelet function is well known in diabetes mellitus. However, the underlying mechanisms are not fully understood. We studied the influence of acute hyperglycaemia on platelet function in nine healthy volunteers before and after reaching a hyperglycaemic steady state (blood glucose: 12·2 mmol/l) using the hyperglycaemic clamp technique. Tests of platelet function were performed in platelet-rich plasma (PRP) using a Born-type aggregometer and ADP to induce aggregation. Platelet sensitivity (PS) to prostacyclin (PGI_2) was evaluated as the amount of synthetic standard necessary to reduce the induced aggregation to 50% (ng/ml PRP). Results: In the euglycaemic state, PS to PGI_2 was 1.38 ± 0.4 ng/ml PRP. After reaching the hyperglycaemic steady state PS to PGI_2 increased significantly (30 min 1.19 ± 0.3; at 60 min 1.16 ± 0.2; at 90 min 1.1 ± 0.2; at 120 min 1.19 ± 0.3; $p <$ 0.05). When normoglycaemia resumed (at 150 min) PS to PGI_2 returned to the basal levels (1.32 ± 0.4). Our results indicate that acute hyperglycaemia does not cause platelet activation. It is unclear which factor in our experimental model induced the improvement of PS to PGI_2. Two concomitant changes could be responsible since plasma insulin increased (from 11.6 ± 4.4 to 62.6 ± 24.2 mU/l) and plasma non-esterified fatty acids decreased (from 0.85 ± 0.3 to 0.25 ± 0.1 mmol/l) during the clamp tests.

176. Prostaglandin E inhibition of glucose-induced insulin secretion in man: evidence for an interference with β-cell calcium stores

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The present study was undertaken to investigate the mechanism by which prostaglandin E (PGE) inhibits glucose-induced insulin secretion in man. Normal volunteers without a family history of diabetes

participated in the study. The infusion of PGE_1 ($0.2\,\mu g \cdot kg^{-1} \cdot min^{-1}$) inhibited the acute insulin response to intravenous glucose, but not to arginine or tolbutamide. An infusion of lysine acetylsalicylate (LAS, 72 mg/min), to block the synthesis of endogenous PGE, caused a marked increase of insulin responses to all the stimulants used. An infusion of verapamil (160 µg/min) did not inhibit the insulin response to glucose in the presence of LAS, but blunted the potentiating effect of LAS on insulin secretion. An infusion of calcitonin (5 U) and somatostatin (500 µg), two agents which interfere with calcium handling in the β cell, inhibited the insulin response to glucose in the presence of LAS. These results indicate that (a) PGE_1 selectively inhibits glucose-induced insulin secretion in man; and (b) the amplifying effect of salicylates on insulin responses to secretagogues is not mediated via an increased calcium entry, but probably reflects a calcium redistribution in the β cell. It is hypothesized that an interference on the mobilization of β-cell calcium stores may explain the negative influence of PGE_1 upon glucose-induced insulin secretion in man.

177. Estimation of β-cell function and the relation to glycaemic control in Type 2 (non-insulin-dependent) diabetes

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The aim of this study was to estimate β-cell function and its metabolic importance in 30 patients with Type 2 diabetes. β-cell function was estimated by measuring fasting plasma C-peptide, C-peptide 6 min after intravenous injection of 1 mg glucagon, the 24-h urine excretion of C-peptide per creatinine excretion and the area under the curve due to the increment in plasma C-peptide ($\Delta AUC \cdot CP$) after a standard meal. We considered the best estimator of β-cell function to be the $\Delta AUC \cdot CP$, since stimulation during a meal represents physiological stimulation of the β cells. Fasting C-peptide and C-peptide 6 min after glucagon both correlated with $\Delta AUC \cdot CP$ after the meal ($r = 0.51$, $p < 0.01$ and $r = 0.46$, $p < 0.02$ respectively), while urinary C-peptide did not. $\Delta AUC \cdot CP$ after the meal correlated with both HbA_1 ($r = -0.47$, $p < 0.02$) and fasting blood glucose ($r = -0.35$, $p < 0.05$). Using fasting C-peptide, C-peptide 6 min after glucagon or urinary C-peptide as estimators of β-cell function, no correlation with HbA_1 or fasting blood glucose was found. HbA_1 correlated with fasting blood glucose ($r = 0.78$, $p < 0.001$). In conclusion, the amount of insulin secreted after a meal is of metabolic importance in Type 2 diabetes. Fasting C-peptide gives the same information on β-cell function as C-peptide 6 min after glucagon. Urinary C-peptide seems to be inapplicable as an estimator of β-cell function in Type 2 diabetes.

178. Islet cell antibodies and HLA-D/DR phenotypes in Type 2 (non-insulin-dependent) diabetes

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Within patients with Type 2 diabetes, a subgroup with secondary oral hypoglycaemic agent (OHA) failure can be regarded as a retarded form of Type 1 (insulin-dependent) diabetes by both presence of cytoplasmic antibodies to islet cells (ICA) and certain HLA-DR phenotypes. This notion is based on the following data: of 67 patients, who had had Type 2 diabetes for at least 1 year, 18 had ICA and 16 of these (89%) developed secondary OHA failure. In contrast, in the ICA-negative subgroup ($n = 49$), 29 patients (59%) developed secondary OHA failure. The time interval between diagnosis of diabetes and development of insulin dependency was significantly shorter in the ICA-positive than in the ICA-negative subgroup (mean ± SEM 3.7 ± 0.9 and 8.4 ± 1.1 years, respectively; $p < 0.01$). By HLA-DR typing a significant excess of DR3 and heterozygous DR3/DR4 phenotypes was found in the ICA-positive patients with secondary OHA failure, akin to that reported in juvenile Type 1 diabetic patients. The heterozygous DR3/W6 phenotype was also significantly increased in the ICA-positive patients, whereas the DR2 and DR5 frequencies were significantly decreased. In contrast, both ICA-negative subgroups with and without secondary OHA failure revealed DR antigen frequencies comparable to the control population.

179. Why is HDL-cholesterol concentration reduced in diabetes?

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To define the reason for reduced plasma HDL-cholesterol levels in diabetes, plasma ^{125}I-HDL kinetics were determined in normal and alloxan (180 mg/kg) diabetic rabbits. Plasma concentrations (mean ± SEM) of glucose (25.2 ± 0.7 versus 6.8 ± 0.03 mmol/l), VLDL-triglyceride (4.7 ± 3.6 versus 0.34 ± 0.07 mmol/l), VLDL-cholesterol (1.24 ± 0.41 versus 0.28 ± 0.08 mmol/l), and LDL-cholesterol (0.67 ± 0.1 versus 0.39 ± 0.08 mmol/l) were higher and HDL-cholesterol was lower (0.31 ± 0.05 versus 0.46 ± 0.10 mmol/l) in diabetic rabbits. Turnover rate of ^{125}I-HDL in plasma was biphasic, and the mean half-time of the first phase was significantly prolonged in diabetic (15.6 ± 1.1 h) compared with control (7.6 ± 1.0 h) rabbits ($p < 0.001$). No differences between the two groups were noted in the turnover rate of the second phase. Insulin treatment of diabetic rabbits reversed the abnormalities in plasma glucose and lipoprotein concentrations, and the half-time of turnover of ^{125}I-HDL (first phase) fell significantly to 3.8 ± 1.0 h ($p < 0.001$). In conclusion, these data suggest that the fall in HDL-cholesterol concentration seen in diabetic animals is secondary to a marked reduction in HDL synthesis. Furthermore, both the abnormalities in lipoprotein concentration typical of diabetes and the defect in HDL kinetics are reversed relatively rapidly by insulin administration.

180. Calcium channel blocker effects on insulin receptor binding in isolated rat adipocytes

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Calcium (Ca^{++}) channel blocker (CB) effects are putatively exerted through inhibition of voltage-dependent Ca^{++} channels. We have reported decreased adipocyte basal (B) and insulin-stimulated (I-S) hexose transport (HT) by verapamil, methoxy-verapamil, diltiazem and nifedipine, while HT is unaffected by depolarizing concentrations of K^+ (60 mmol/l) and $^{45}Ca^{++}$ uptake remains unchanged by the above Ca^{++} CB. The mechanism of HT inhibition by Ca^{++} CB was assessed with steady-state binding studies of ^{125}I-monoiodoinsulin to adipocyte plasma membranes at pH 7.6 and 20 °C for 2 h. B/F ratios at 5 pmol/l ^{125}I-insulin and 100 µg plasma membranes protein were 0.137 ± 0.012 for controls and 0.130 ± 0.015 (NS) in the presence of 1.25 mmol/l verapamil ($n = 5$). B/F ratios for controls with 2% DMSO, the solvent for the other Ca^{++} CB, were 0.075 ± 0.006, which decreased significantly with 0.5 mmol/l methoxy-verapamil (0.053 ± 0.005, $p < 0.05$), 0.5 mmol/l diltiazem (0.056 ± 0.005, $p < 0.05$) and 0.16 mmol/l nifedipine (0.057 ± 0.004, $p < 0.05$) ($n = 5$). The data indicate that (1) voltage-dependent Ca^{++} channels do not mediate Ca^{++} influx into rat adipocytes and (2) Ca^{++} channel blocker inhibition of basal and insulin-stimulated hexose transport involves both insulin receptor and post-receptor mechanisms.

181. Data processing in a diabetic clinic

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Since 1926 the Portuguese Diabetic Association has had an outpatient clinic and to date 20000 patient files are on record. In 1982 we started to organize a database with this information using an ABC-26DW microcomputer with storage capacity of 8 Mb. The programme created for the introduction, updating and retrieval of information uses a screen-oriented editor to facilitate operation and limit mistakes. For each patient, besides data for administrative purposes, we register the type of diabetes, physical activity, latest observations and in the case of death, year and cause. The clinical picture is defined according to pre-established criteria for: personal history (10 entries), family history (9), previous eating habits (4) and present dietary programme (4), therapy (3), compensation (3), co-existing diseases (13), cardiovascular parameters (7), ophthalmological situation (3) and other complications (14). Implementation of the system implied uniformity of clinical data collection, with common semantics for clinical staff and data operators. The clinical picture is updated whenever a patient comes to the clinic. Some data are put on record separately for each visit to allow easier study of changes with time. Many possibilities of clinical research and statistical analysis are created by this system.

182. Effect of sorbinil on lens metabolites in experimental diabetes

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Sorbitol, fructose and glycerol-3-phosphate accumulate in high concentration in the lens of untreated diabetic rats. In contrast, the lens content of glutathione, glutamate and ATP decreases and there is a slowing of lens growth. The aim was to examine the effect of an aldose reductase inhibitor, sorbinil (Pfizer, UK), on the above metabolites in relation to reversal of these changes in short-term (after 1 week) and long-term (after 1 month) of uncontrolled diabetes. Treat-

ment with sorbinil (10 mg/kg body weight) was highly effective in lowering lens sorbitol in both short- and long-term diabetes. Values for the five groups: (A) control, (B) diabetic 2 weeks, (C) diabetic 1 week + sorbinil 1 week, (D) diabetic 2 months, (E) diabetic 1 month + sorbinil 1 month, were respectively 0.18 ± 0.04, 11.0 ± 1.1; 1.30 ± 0.08; 14.5 ± 1.2, and 4.15 ± 0.04 μmol/g (mean ± SEM). The values for fructose were: 0.87 ± 0.15; 7.50 ± 0.31; 4.20 ± 0.22; 9.1 ± 0.5 and 6.8 ± 0.4 μmol/g respectively. In contrast, lens glutathione and glutamate, which both fell in diabetes to approximately 25% and 40% of control values, were restored towards the normal level by sorbinil treatment in both short- and long-term diabetes. A parallel restoration in lens weight and protein content was noted following long-term sorbinil treatment (groups D and E). Thus sorbinil appears to influence metabolites distal to its primary site of action.

183. Insulin action remains improved 6 weeks after withdrawal of insulin therapy in Type 2 (non-insulin-dependent) diabetes

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The improvement in insulin action induced by 4 weeks' insulin treatment with dietary supervision and weight maintainence has been studied 6 weeks after insulin withdrawal. Seven Type 2 diabetic patients (mean ± SD: body mass index 30.0 ± 5.8 kg/m^2, age 50.9 ± 6.4 years, fasting plasma glucose 14.7 ± 2.3 mmol/l) underwent glucose clamps at insulin infusion rates (IIR) of 40 and 400 mU · m^{-2} · min^{-1} before (T1), immediately after 4 weeks insulin treatment (T2), and (in five patients) 6 weeks following insulin withdrawal (T3). In the successful clamps, at T2 metabolic clearance rate of glucose, measured at similar plasma glucose concentrations, had increased at the low IIR from 1.48 ± 0.61 to 2.69 ± 1.11 ml · kg^{-1} · min^{-1} ($n = 6$; mean change + 100.3%, range − 12 to + 22%); and at the high IIR from 6.17 ± 1.69 to 15.33 ± 5.03 ml · kg^{-1} · min^{-1} ($n = 6$; mean change + 151.8%, range + 77% to + 266%). At T3, at the low IIR, three of the four subjects studied retained some improvement in insulin action (metabolic clearance rate of glucose 1.38 ± 0.35 ml · kg^{-1} · min^{-1} at T1 versus 2.59 ± 1.36 ml · kg^{-1} · min^{-1} at T3; mean change + 82.0%, range − 4 to + 266%); and at the high IIR five subjects studied retained some improvement from 6.11 ± 1.89 ml · kg^{-1} · min^{-1} at T1 versus 7.91 ± 1.98 ml · kg^{-1} · min^{-1} at T3 (mean change 35.2%, range + 18% to + 72%). Five control diabetic patients (body mass index 36.1 ± 4.4 kg/m^2, age 56.8 ± 7.8 years, fasting plasma glucose 13.9 ± 2.2 mmol/l) underwent the same dietary protocol but did not receive insulin treatment; their metabolic clearance rate of glucose at both IIR remained unchanged between T1 and T2. The improved insulin action induced by 4 weeks' insulin treatment of Type 2 diabetes without weight loss can still be demonstrated 6 weeks after insulin withdrawal.

184. Prolonged insulin release from pancreatic β cells with surface-bound and intracellular glibenclamide

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Purified β-cells were employed to investigate the mechanisms leading to the prolonged insulin secretory activity which is characteristically observed after glibenclamide exposure. At 5.6 mmol/l glucose, glibenclamide and tolbutamide elicited comparable biphasic insulin release patterns: this secretory response was similar to that measured at 20 mmol/l glucose. After drug removal, only the stimulatory effect of glibenclamide persisted for at least 1 h, while the cells remained almost completely refractory to glucose. This prolonged secretagogue action of glibenclamide appeared unrelated to the rate of glucose oxidation or cyctic AMP formation, but was associated with a slow disappearance of the hypoglycaemic agent from the pancreatic β cells. The subcellular localization of the drug that remained incorporated after extracellular removal was determined on autoradiographs of ^{3}H-glibenclamide-treated β cells. These data illustrate that glibenclamide remained not only bound to the plasma membrane, but was also internalized by the cells. It is so far unknown whether this intracellular localization corresponds to a disposal process or to a functionally relevant compartment.

185. Direct insulin effect on myocardial calcium uptake in the streptozotocin-diabetic rat

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Cardiac pump failure is often seen in diabetic patients. A decreased catecholamine sensitivity might be responsible and, as transsarcolemmal calcium transport determines the contractile state of the myocardial cells, we studied the myocardial calcium uptake in response to isoproterenol (ISO, 10^{-4} mol/l) in isolated perfused hearts from short-term streptozotocin-(STZ)-diabetic rats. Radiolabelling followed by cold washout gave the following results in mol/min perfusion per g protein: basal level: control (C) 125.54 ± 4.18 (SEM); diabetes (D) 123.75 ± 5.11, C + ISO 163.44 ± 7.50 ($2p < 0.0001$). Twenty-four hours after STZ 165.33 ± 21.46, 48 h: 153.75 ± 6.33, 72 h: 163.31 ± 15.60, 96 h: 121.69 ± 7.54 ($2\,p < 0.01$), 8 days: 121.08 ± 4.10 ($2\,p < 0.01$). Thus, 96 h after STZ when hyperglycaemia had persisted for 72 h, the ISO-induced calcium uptake decreased significantly. Insulin added to the perfusate (0.1 U/ml) increased the ISO-response in these diabetic hearts (138.65 ± 6.43, $2\,p < 0.05$) and a total normalization was seen after insulin in vivo (10 U/kg intraperitoneally) 60–90 min before perfusion (150.30 ± 4.82). β-receptor blockade (propranolol, 10^{-5} mol/l) abolished the ISO-response and the excessive calcium after ISO was equally distributed throughout the individual heart segments. Thus, insulin has an acute direct effect on ISO-induced calcium transport and abnormalities in the sarcolemma might possibly be responsible for the increased incidence of pump failure seen in diabetic patients in stressful situations, i. e. after myocardial infarction.

186. Glycated proteins in diabetic and hypoglycaemic animal models

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Stable glycated (glycosylated) haemoglobins and plasma proteins were determined by affinity chromatography using glycogel B in animal models. Adult Aston *ob/ob* mice and C57BL/KsJ *db/db* mice exhibited 1.5–1.9-fold increases of body weight, 2.5–3.4-fold elevations of plasma glucose and 20.9–29.3-fold elevations of plasma insulin when compared with lean controls. Glycated haemoglobins and plasma proteins were raised 7.2–8.2-fold and 6.6–6.7-fold respectively. In adult NEDH rats administration of streptozotocin resulted in insulin deficiency (5.9-fold decrease) and hyperglycaemia (3.2-fold increase) by 2 days. Glycated plasma proteins increased significantly in 2 days by 1.2-fold, followed by glycated haemoglobins (1.6-fold increase) after 8 days. In contrast, implantation of transplantable insulinoma fragments produced hyperinsulinaemia (3.3-fold increase) and hypoglycaemia (1.7-fold decrease) by 15 days. Glycated plasma proteins and haemoglobins decreased (2.4-fold and 2.2-fold respectively) 7 days before demonstration of hypoglycaemia. Good correlations were observed in mice and rats between glucose and both glycated haemoglobins ($r = 0.92$ and $r = 0.87$, respectively) and glycated proteins ($r = 0.85$ and $r = 0.92$). The results show that the measurement of glycated blood proteins by affinity chromatography provides a sensitive and reliable indicator of the recent glycaemic environment in animal models.

187. Hyperosmolar coma and thrombosis: is sodium responsible?

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Diabetic patients in hyperosmolar coma are at risk of thrombosis. This has been attributed both to dehydration and hyperviscosity. We investigated the effect of hypernatraemia on factor VIII (FVIII), euglobulin clot lysis activity and fibrinopeptide A (FPA) generation time by infusing hypertonic saline in 11 male volunteers. Following infusion of 450 ml of 5.8% saline, plasma osmolality rose from a mean of 287–302 mosm. There was no change in mean FVIII C assayed by one-stage method $75 \pm 5.5\%$. However, there was a highly significant fall in FVIII C (two-stage) (mean ± SEM) from $80 \pm 4.5\%$ to $42 \pm 5.5\%$, $p < 0.002$). The FVIIIRag:C ratio rose from 0.92 ± 0.11 to 2.16 ± 0.59 ($p < 0.05$), after 4 h. FPA generation time shortened progressively from 7.2 ± 0.4 min before infusion to 5.4 ± 0.6 min after 2 and 5.3 ± 0.6 min after 4 h ($n = 6$). Euglobulin clot lysis activity (10^6/ELT^2) rose from 62 ± 20 to 370 ± 45 unit after 3 h. These results indicate that a sodium-induced increase in plasma osmolality is accompanied by changes in haemostatic function consistent with hypercoagulability. This mechanism could contribute to the pathogenesis of the thrombo-embolic complications of hyperosmolar states in diabetes mellitus.

188. Monoclonal antibodies raised against biosynthetic human proinsulin

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Circulating proinsulin is heterogenous, consisting probably of intact proinsulin and two intermediates hydrolysed at 65/A1 and 32/33. Ex-

isting assay methodologies do not distinguish between the various forms. To develop more specific and sensitive assays we have raised monoclonal antibodies against biosynthetic human proinsulin. Two antibodies bind the insulin moeity of proinsulin and two bind only to proinsulin and not to insulin or C-peptide. Both insulin-binding antibodies bind to 65/A1 and 32/33 more avidly than intact proinsulin or insulin. The affinity constants for these antibodies are 1.2×10^{10} and 3.2×10^{10} l/mol respectively. The first of the proinsulin specific antibodies does not bind to proinsulin hydrolysed at 32/33, thus suggesting that the antibody binds to the region where the β-chain of insulin joins with C-peptide. The affinity constant of this antibody is 6.3×10^{11} l/mol. The second proinsulin-specific antibody is of low affinity (4.1×10^{8} l/mol) and reacts only with intact proinsulin. Use of the insulin-proinsulin binding antibodies in a two-site assay with labelled proinsulin-specific antibodies enables us to measure specifically intact proinsulin, 65/A1 and 32/33.

189. Vasoactive intestinal peptide inhibits insulin-stimulated glucose transport in adipocytes

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Vasoactive intestinal polypeptide (VIP)-secreting tumours produce hyperglycaemia. We studied the effects of VIP on insulin action in rat adipocytes. Insulin (600 mU/l) caused a 15-fold increase in glucose transport, measured by 2-deoxyglucose uptake. VIP inhibited insulin-stimulated glucose transport completely (IC_{50} 10 µg/l) but only when adenosine (which is released by adipocytes) was prevented from accumulating by addition of adenosine deaminase (ADA; 2 U/ml). VIP did not inhibit ^{125}I-insulin binding, demonstrating that the effect is distal to the insulin receptor. ^{125}I-VIP bound specifically to adipocytes, reaching equilibrium by 2.5 h at 16 °C. Binding was inhibited by unlabelled VIP (IC_{50} 10 µg/l) but not by 1 mg/l glucagon, growth hormone or insulin. VIP (2 mg/l) increased cellular cyclic AMP levels (measured in the presence of 1 mmol/l aminophylline) from 1.0 to 10.4 pmol/10^5 cells in the absence of ADA, and from 1.2 to 22.8 pmol/10^5 cells in the presence of ADA. This suggests that VIP activates adenylate cyclase, and that adenosine can restrain the response. In summary (1) VIP binds specifically and with high affinity to adipocytes, (2) VIP increases cyclic AMP levels and inhibits insulin-stimulated glucose transport. In conclusion, VIP inhibits insulin action in adipocytes. This finding may explain the diabetogenic action of VIP.

190. Effect of insulin secretagogues on prostaglandin E_2 levels in isolated rat islets of Langerhans

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Prostaglandin synthesis inhibitors stimulate insulin release in vitro. Our aim was to seen whether other stimuli for insulin secretion lower islet prostaglandin E_2 (PGE_2) levels. Groups of 20 rat islets were incubated with test substances in 1 ml of medium. After 20 min the PGE_2 and secreted insulin were radioimmunoassayed; islet tissue PGE_2, protein, and latterly cyclic AMP levels were measured also. PGE_2 secretion reflected its synthesis and was typically 4–5 pg PGE_2/µg islet protein. Significant reductions in PGE_2 secretion induced by 20 mmol/l glucose to 38%, 5 mmol/l arginine (63%), 5 mmol/l leucine (72%), 6 mmol/l glyceraldehyde (64%), 1.25 mmol/l sodium salicylate (63%), 28 µmol/l indomethacin (24%) and 50 µg/ml 'Flurbiprofen' (40%) were accompanied by stimulation of insulin release. However, 20 mmol/l glucose plus 5 mmol/l theophylline, 15 µmol/l sodium arachidonate and 10 µg/ml 'Frusemide' stimulated insulin release very significantly while tending to raise islet PGE_2. Sodium salicylate had little effect on islet cyclic AMP compared with its stimulatory effect on insulin secretion, whereas 10^{-5} mol/l exogenous PGE_2 blocked insulin release, but produced a sevenfold elevation in islet cyclic AMP. We conclude that PGE_2 effects on insulin secretion can be dissociated from changes in cyclic AMP and that in several, but not all cases, there is a negative correlation between islet PGE_2 production and insulin secretion.

191. A simple diabetic clinic information system

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A simple form, based on a modified conventional manual system, is used for managing the records of a diabetic clinic in a district general hospital. Nurses record the routine data. Much space is left clear for free text comments. The clinical team meets regularly and using the forms checks them and reviews the progress and management of each patient. Selected items of data are recorded in a computer system to provide a simple diabetic database. Three photocopies of each of the forms are made: one is sent to the general practitioner, avoiding routine letters; one is placed in the patient's notes; the third is saved in case the patient's main hospital notes are unavailable, and as a source of data for research. Monthly the computer produces tables and lists giving basic information on the state of the clinic. Furthermore, *ad hoc* reports are frequently made to answer specific questions. This system has simplified the management of the clinic, is easily used by medical staff inexperienced in the use of computers, and causes minimal increase in the clinical workload. The database has facilitated a number of research studies.

192. Long term review of renal transplantation in diabetic patients

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During the decade 1974–1984, 80 renal transplants have been performed in 68 diabetic patients. Of 28 patients (22 with nephropathy, six with non-diabetic renal disease) who survived more than 2 years, satisfactory renal function (serum creatinine <200 µmol/l) was observed in 15 patients (11 with neuropathy, 4 with non-diabetic renal disease) for a median period of 4 years (range 3–9 years). In seven other patients, serum creatinine increased over 1–6 years due to episodes of rejection; of second transplants performed in four of these patients, two have been successful. The grafts failed rapidly (<3 months) in the remaining six patients who have managed well on haemodialysis for 3–6 years with one death. Five patients died 2.2–4.7 years after transplantation, two with functioning grafts. Of the 28 patients, 19 were fit (13 employed). Four patients were blind (three having been so at transplantation). Amputations were performed on five patients (two below-knee, one mid-tarsal, two digits). These results suggest that successful renal transplantation can be achieved in unselected diabetic patients. However, some patients were disabled by diabetic complications. Early transplantation before the need for dialysis, together with careful case selection in patients referred late, may be important in reducing morbidity and mortality.

193. Binding- and kinase-defective insulin receptor in monozygotic twins discordant for Type 2 (non-insulin-dependent) diabetes

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Identical human twins are a unique resource for evaluating aetiological importance of non-genetic factors in Type 2 diabetes. In the present study, four identical twin pairs discordant for Type 2 diabetes or impaired glucose tolerance were used to evaluate factors affecting binding and kinase properties of the insulin receptor. Insulin binding assay performed on circulating erythrocytes and monocytes showed a 10–40% deficit in binding in all affected patients compared with their normal co-twins. In all cases the binding defect consisted of reduced affinity with no change in receptor number. This defect was also retained in the solubilized receptor preparations. In two of these pairs, the protein kinase activity of the erythrocyte insulin receptor was studied. For both, maximal insulin-stimulated phosphorylation of the β-subunit of the receptor was similar in affected and non-affected twins. However, the dose-response curve of phosphorylation was shifted to the right, consistent with the affinity change in the receptor binding. Thus, Type 2 diabetes is associated with an alteration in both receptor binding and kinase activation. These data suggest that both of these defects are acquired, and not inherited.

194. Increased number of glucose transport systems in adipocytes from young hyperinsulinaemic obese Zucker rats

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It has been proposed that impaired translocation of microsomal glucose transport systems (GTS) to plasma membrane in adipocytes resistant to insulin in terms of glucose transport (GT) was the consequence of a relative depletion of GTS in the intracellular pool. We examined the concentration of GTS in inguinal adipocytes from young (30 day-old) hyperinsulinaemic obese Zucker rats, where GT is hyper-responsive to insulin compared with lean rats. GT activity was assessed by using 3-0-methyl-glucose, (6 ^{14}C) glucose or 2-deoxyglucose (0.05 mmol/l). GTS were quantified by measuring specific D-glucose inhibitable cytochalasin B binding in plasma and microsomal

membranes of adipocytes pre-incubated ± insulin (370 mU/l). Without insulin, GTS/mg protein in both membrane fractions of obese cells was nearly double that of lean rats. Insulin increased GT by 2 fmol · cell^{-1} · min^{-1} in obese versus 0.3–0.8 in lean adipocytes according to the tracer used. Correlatively, the estimated number of GTS appearing in the plasma membrane in response to insulin was about fivefold higher in obese than in lean adipocytes; GTS were translocated from a 13-fold higher intracellular pool. Whether increased GTS concentration in adipocytes of Zucker obese rats results from the genetic lesion directly or through hyperinsulinaemia remains to be answered. This model supports the role of an intracellular GTS pool in the modulation of insulin action on GT.

195. Heterotopic segmental pancreatic transplantation can provide good metabolic control in Type I (insulin-dependent) diabetes

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Since November 1981 we have performed 19 combined kidney and pancreas transplantations in 18 uraemic Type I diabetics patients. Vascular anastomoses were made to the iliac vessels, thus insulin was delivered into the systemic circulation. Exocrine secretion was diverted to the gastro-intestinal tract. Six patients have currently functioning grafts (longest follow-up 28 months) with essentially normal glycaemic control as indicated by mean values for fasting blood glucose, haemoglobin A_1 and intravenous insulin tolerance test k-values of 4.8 mmol/l, 7.7% and 1.3%/min, respectively. In four recipients glucose metabolism was evaluated in more detail by the hepatic venous catheter technique 6–19 months after operation. The arterial fasting concentration of glucose was slightly lower in recipients than in healthy controls ($n=7$) (3.8 ± 0.2 versus 4.6 ± 0.1 mmol/l, $p<0.01$) while higher values were recorded for glycerol (84 ± 12 versus 52 ± 4 μmol/l, $p<0.05$), immunoreactive insulin (26 ± 3 versus 9 ± 1 mU/l, $p<0.001$) and immunoreactive glucagon (199 ± 10 versus 77 ± 13 ng/l, $p<0.001$). Other, metabolite levels (lactate, pyruvate, β-hydroxybutyrate, acetoacetate, non-esterified fatty acid, amino-acids) were similar in patients and controls. Hepatic glucose production and splanchnic exchange of gluconeogenic precursors were similar also. In response to a small intravenous glucose infusion (2 mg/kg for 45 min) hepatic glucose output was inhibited identically in patients and controls. It is concluded that heterotopic pancreatic transplants with systemic insulin delivery can provide adequate metabolic control in Type I diabetes.

196. Metabolic patterns in sand rats *(Psammomys obesus)*

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Sand rats from a colony maintained on laboratory chow ad libitum were divided into four groups on the basis of plasma glucose and insulin levels (group A = normoglycaemic, normoinsulinaemic; group B = normoglycaemic, hyperinsulinaemic; group C = hyperglycaemic, hyperinsulinaemic, group D = hyperglycaemic, insulin deficient). Body weight was positively correlated with insulin levels. In all sand rats fatty acid synthesis of adipose tissue was negligible. Basal glucose uptake of isolated strips of soleus muscle was similar to that of albino rats and was not significantly different in groups A–D, whereas the response to insulin was lowest in group C. In vivo administration of labelled VLDL and 2-deoxyglucose (2-DG) showed a progressive increase in VLDL uptake into muscle in groups B and C (20% and 60%, respectively), whereas 2-DG uptake was reduced to 40% and 20%, respectively. In adipose tissue 2-DG uptake showed a similar pattern, whereas VLDL uptake was not affected by hyperinsulinaemia (group B), but was reduced in hyperglycaemic animals (groups C and D). Changes in activity of adipose tissue and muscle lipoprotein lipase were closely correlated with VLDL uptake. It is suggested that the development of obesity and hyperglycaemia is a result of the dissociation between the effects of insulin resistance on glucose utilization by muscle and on VLDL uptake by adipose tissue.

197. Evidence for a direct, non-paracrine effect by glibenclamide on glucagon secretion from the perfused rat pancreas

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Perfusion of the isolated rat pancreas with glibenclamide (1 μg/ml), at basal glucose (3.3 mmol/l) and at physiological calcium levels (2.6 mmol/l), elicits insulin and somatostatin responses, whereas glucagon release remains unaffected by the drug. Presently we demonstrate that the interaction of the compound with islets was dependent on calcium concentration in the perfusate. At 0.6 and 0.25 mmol/l of calcium, insulinogenic effect of glibenclamide was enhanced but its stimulatory effect on somatostatin was abolished. In addition, at 0.6 mmol/l calcium, the compound suppressed glucagon release by 70%. The latter effect could be accounted for by direct interaction of glibenclamide with A cells or by the paracrine effect of insulin or by both. In pancreases from alloxan-diabetic rats, glibenclamide completely lost its inhibitory action on glucagon release, which was not restored by adding insulin (25 U/l) to perfusion media during administration of the compound. However, in pancreases isolated from diabetic rats treated with insulin for 3 days, glibenclamide suppressed glucagon release – provided that a low calcium medium was used – although insulin and somatostatin release were lacking. To conclude, at low calcium concentrations, glibenclamide suppresses glucagon secretion by direct action on the A cell and not through paracrine interactions by insulin or somatostatin. Prolonged insulin deficiency impairs the action of the compound.

198. Thermal sensory loss in diabetic neuropathy

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Small unmyelinated nerve fibres carry thermal and sympathetic impulses. We have assessed small and large fibre function (thermal and vibration sensation respectively) in diabetic patients with neuropathy and have shown that small fibre defects are more common. Hands and feet were examined using a Marstock device for thermal discrimination and a Biothesiometer for vibration sensation in 47 diabetics (mean age 37 years, range 22–55 years; mean duration of diabetes 17 years, range 3–42 years) and 54 control subjects (mean age 34 years; range 21–55 years). Of the diabetics, 32 had either neuropathic foot ulceration, Charcot joints and/or autonomic symptoms (group 1) and 15 neuropathic pain (group 2). In group 1, 31/32 had abnormal thermal sensation (mean 20.8 ± 1.5 °C, controls 5.9 ± 1.3 °C, $p<0.001$) and 27/32 abnormal vibration sensation (mean 27.5 ± 1.9 volts, controls 5.6 ± 1.3 volts, $p<0.001$); 23/32 abnormal thermal sensation and 19/32 abnormal vibration sensation in the hands. Sometimes extreme thermal defects occurred with entirely normal vibration. Painful neuropathy cases (group 2) showed a range of sensations from normal to severely abnormal for both modalities, but only thermal sensation was significantly impaired. These results indicate that small fibres are particularly vulnerable in diabetic patients and could account for the sympathetic nerve failure and concomitant increased foot blood flow characteristic of diabetes.

199. Metabolic control and psycho-social implications of continuous subcutaneous insulin infusion in 54 non-highly selected Type 1 (insulin-dependent) diabetic patients followed by routine conditions

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Our aims were to evaluate the clinical and psycho-social implications of continuous subcutaneous insulin infusion (CSII) in 54 Type 1 diabetic patients, followed on a routine basis. Patients were studied for two periods of 4 months, one with a conventional treatment (2–3 injections, self-blood glucose monitoring) and the other with CSII. We studied metabolic control (daily values of blood glucose strips, urine analysis, insulin reactions, glycosylated haemoglobin), treatment acceptability by patients and patient's relatives, psychological profile. We also recorded all the unexpected phone-calls. Among the initial cohort, 38 patients completed the study, seven dropped-out and nine interrupted CSII, mainly for cutaneous intolerance. During CSII, patients noted non-redhibitory disturbances concerning sleep (30%), sex (68%), clothing (26%). The main avantages were dietary liberalization, less insulin reactions and a greater feeling of well-being. Pump acceptability was always good even in patients who did not wish to pursue CSII. Patients keeping the pump had no particular psychological profile; they had more late complications ($p<0.05$), were more often non-smokers ($p<0.05$), had a tendency to a greater sedentary attitude and were previously treated by three daily injections.

200. Protein kinase activity of the insulin receptor from muscle of normal rats and of diabetic Zucker rats

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The 95 K sub-unit of the insulin receptor contains tyrosine kinase activity. This was shown for receptors of hepatocytes, adipocytes and a

number of other tissues which are not primary targets of insulin action. We studied receptor kinase in another target tissue, muscle, and compared receptor kinase of insulin -resistent diabetic Zucker rats with the kinase of normal rats. Insulin binding and insulin action on glucose transport were determined in isolated cardiomyocytes. The receptor was solubilized from cardiomyocytes or from skeletal muscle by Triton X 100 and was enriched by wheat germ affinity chromatography. Phosphorylation was studied with γ-[^{32}P]ATP and insulin and the receptor was identified by immunoprecipitation with receptor antibody, PAGE and autoradiography. Insulin stimulated receptor phosphorylation was 5-fold (ED_{50} 3×10^{-9} mol/l). The effect was detectable at 10^{-10} mol/l, parallel to the effect on glucose transport. The kinase from muscle phosphorylates on tyrosine, is active on exogenous substrate and is active after previous immunoprecipitation. Thus, receptor kinase from muscle has similar characteristics to that found in the other target tissues. If skeletal muscle from diabetic Zucker rats is used, similar insulin responsiveness of the kinase is found, while its insulin sensitivity is decreased. This might contribute to the pathogenesis of insulin resistance in this animal model of non-insulin-dependent diabetes.

201. A new technique for comparing the fate of glucose when given via three different routes

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Some animals have a normal intravenous glucose tolerance test but glucose intolerance during an oral glucose tolerance test. This prompted us to set up a new technique: the 'clamp' techniques are adapted to normal anaesthetized rats with these modifications: *Step 1:* a needle is inserted in the post-pyloric duodenum; *Step 2:* another in the portal vein; *Step 3:* a double catheter in a jugular vein; *Step 4:* a catheter in a carotid artery (blood sampling). Needles or catheters are connected to pumps for unlabelled glucose (steps 1–2–3) and labelled glucose infusion (step 3) enabling us to calculate glucose metabolism and hepatic glucose production. *Strategy:* step 1 mimicks glucose fate and insulin response during ingestion; step 2 eliminates gut factors in such a fate; step 3 is a mirror of peripheral hormono-metabolic changes. Realization of steps 1–2–3 requires 1 h. After a further 30 min rest, labelled glucose is infused and a plateau of specific activity obtained 1 h later. Subsequently, plasma glucose concentrations and specific activity are measured every 15 min for 2 h. During this period glucose metabolism and hepatic glucose production remained stable (1.53 ± 0.05 and 1.55 ± 0.05 mg/min, respectively, $n = 8$). In conclusion, hyper- and/or euglycaemic clamps can now be applied under conditions that will permit determining the fate of glucose while entering the internal milieu via the three routes mentioned.

202. Dissociation between phosphatidylinositol hydrolysis and insulin secretion of isolated mouse pancreatic islets

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In several exocrine and endocrine cell types, stimulated secretion induces rapid hydrolysis of phosphatidylinositol (PI) to diacylglycerol and inositol phosphate. Re-synthesis of PI then occurs after formation of phosphatidic acid. The impact of this PI-response on 'stimulus-secretion' coupling remains obscure. In most previous islet studies, stimulated PI turnover has been estimated indirectly by radioactive labelling of re-synthesized PI. In this study, the rate of PI-turnover was directly determined by measuring the initial breakdown of PI when challenging the islets with different secretagogues. Exposure of mouse islets prelabelled with ^{3}H-glycerol or ^{3}H-inositol to carbamylcholine (10^{-4} mol/l) for 30 min increased the rate of insulin secretion and was accompanied by a significant disappearance of PI-bound radioactivity. This PI response was also present during carbamylcholine stimulation in a calcium-free medium, which blocked insulin secretion. Insulin release induced by glucose (16.7 mmol/l), arginine (10 mmol/l), theophylline (5 mmol/l) or a high K^+ concentration (25 mmol/l) was, on the other hand, not associated with a concurrent PI breakdown. The results indicate that hydrolysis of PI in pancreatic islets might be a phenomenon unrelated to stimulated insulin secretion. Thus, a previously suggested correlation between PI hydrolysis and glucose-stimulated insulin secretion may need reconsideration.

203. Effects of adrenaline on lipolysis and fatty acid re-esterification in isolated human adipocytes

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In rat adipose tissue it has been reported that adrenaline increases lipolysis, at the same time increasing fatty acid (NEFA) re-esterification, implying an increased cycling rate between triglyceride and NEFA. By contrast, glycerol phosphate acyltransferase activity is decreased by adrenaline, suggesting that NEFA esterification would be inhibited. The situation in man is equally uncertain; we have therefore studied the effect of adrenaline on the triglyceride/NEFA cycle in isolated human adipocytes. Adipocyte incubations were performed for 4 h, in the presence of 10^{-10} to 10^{-5} mol/l adrenaline. Lipolysis was assessed as the total change in glycerol release, and NEFA re-esterification as the divergence of the NEFA: glycerol molar ratio from 3:1. The threshold for stimulation of lipolysis was 10^{-9} mol/l adrenaline, and lipolysis increased further between 10^{-9} and 10^{-6} mol/l. At 10^{-6} mol/l adrenaline, lipolysis increased 2–10 fold over basal levels. In the absence of adrenaline 35% of NEFA released were re-esterified. Adrenaline at 10^{-6} mol/l inhibited NEFA re-esterification completely. Thus, in human adipocytes adrenaline increases lipolysis and inhibits NEFA re-esterification; these results are similar to those previously reported with noradrenaline. Substrate cycling between triglyceride and fatty acid is therefore decreased or abolished by the catecholamines.

204. C-peptide glucose and insulin levels following glucose: the contribution of the enteroinsular axis to insulin secretion

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Peripheral insulin concentrations are greater after oral than intravenous glucose, due, according to recent studies, to increased hepatic extraction of insulin rather than increased insulin secretion. The workers failed to show a parallel increase in C-peptide and insulin with oral rather than intravenous glucose. However, these studies measured venous glucose although the β cell responds to arterial glucose. We measured peripheral venous plasma insulin and C-peptide in 10 healthy subjects, given either 100 g glucose orally or sufficient intravenous glucose to produce similar glucose concentrations measured in peripheral arterialised blood. Both plasma insulin and C-peptide levels were greater following oral compared to intravenous glucose (mean $\pm$ SEM area under insulin curve 0–180 min (5638 ± 904 versus 1987 ± 433 mU·l^{-1}·min^{-1}, $p < 0.0025$; area under C-peptide curve 891 ± 112 versus 386 ± 59.4 mg·l^{-1}·min^{-1}, $p < 0.0005$). Arteriovenous plasma glucose differences were greater following oral than intravenous glucose and correlated positively with plasma insulin concentrations ($r = 0.94$, $p < 0.0025$). These results suggest that the main cause of increased peripheral insulin levels following a large carbohydrate load is augmented insulin secretion, in accordance with the entero-insular axis concept. They emphasise the need to measure both arterial and venous glucose concentrations for the correct interpretation of experiments investigating glucose homeostasis.

205. Induction of diabetes in the Bio-breeding/Worcester (BB/W) rat with conditioned medium

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BB/W rats develop spontaneous autoimmune diabetes between 60–120 days of age. Diabetes prior to 60 days of age occurs with a frequency of $< 0.5\%$. Diabetes can be adoptively transferred by culturing splenic lymphocytes of acutely diabetic rats with concanavalin-A (Con-A) and administering the cells to young diabetes-prone (DP) rats. Diabetes can also be transferred by this method to diabetes-resistant ("W-line") BB rats and Wistar Furth (WF) rats pre-treated with immunosuppression. To study the mechanism of transfer, rats (aged 23–28 days) were given intraperitoneal injections (1.0 ml/day for 4 weeks) of conditioned media from 72 h incubations of Con-A (2.5 µg/ml) treated splenocytes ($1\text{–}2 \times 10^6$/ml) collected from acutely diabetic donors. Diabetes occurred in 19% (3/16) of DP recipients before age 60 days. In addition, 28% (2/7) of resistant W-line rats developed acute diabetes after identical treatment. Media prepared from spleen cells from WF rats, the ancestor of the BB mutation, induced diabetes in 50% (7/14) of DP recipients prior to 60 days of age. Injections of Con-A containing unconditioned medium are ineffective. We conclude that a soluble substance given frequently can induce diabetes in both diabetes-prone and resistant (W-line) BB rats. Studies are underway to determine the specificity and chemistry of the factor(s) involved and the mechanism of transfer.

206. The average molecular weight of insulin in solution

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The spatial configuration of insulin in zinc insulin crystals has been thoroughly studied, but knowledge about the molecular arrangement of insulin in solution is limited. This molecular state is important for bioavailability, immunogencity and timing, and there is a need to characterize insulin in the injection solution and after injection. Commonly used analyses for characterizing proteins in solution have limitations: sample dilutions, removal of additives, and denaturation (e.g. in use of reverse-phase high-pressure liquid chromatography, gel chromatography or the ultracentrifuge) accentuate some molecular characteristics and exclude others. To describe the physico-chemical state without any external interference, mol.wt. of insulin in solution has been estimated using low-angle laser-light scattering, a direct measurement without calibration and Zimm plot. Mol. wt. of insulin in 100 IU/ml has been estimated as 150000 dalton (contrary to previous results obtained employing gel chromatography). Under disaggregating conditions and after removal of the zinc considerably lower mol.wt. was found. Measurement of mol.wt. of insulin from different species in a series of buffer solutions will be presented and discussed.

207. Decreased plasma glucagon response to arginine infusion and increased plasma somatostatin in Type 1 (insulin-dependent) diabetic patients without residual β-cell function

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Recent studies suggest that some islet cell surface antibodies react with A-cells. Glucagon response to insulin-induced hypoglycaemia is defective in long standing Type 1 diabetes. We therefore studied plasma glucagon and plasma somatostatin responses to arginine in long-term Type 1 diabetes without B-cell function ($n=13$) and controls ($n=13$). Plasma glucagon increased from 27 ± 4.7 to 176 ± 23.1 pmol/l (20 min) in Type 1 diabetes, in controls from 36 ± 5.0 to 302 ± 31.9 pmol/l (20 min; $p<0.01$). Plasma somatostatin increased from 24.2 ± 2.5 to 31.1 ± 3.9 pmol/l at 10 min in the diabetic group, in controls from 17.9 ± 1.7 to 23.9 ± 3.4 pmol/l at 10 min ($p<0.01$). The plasma glucagon concentrations were significantly lower and the plasma somatostatin concentrations were significantly higher in the diabetic patients. We then treated 42 Type 1 diabetic patients without residual B-cell function, randomly assigned to three different insulin treatment regimens (twice a day; multiple injections; CSII), for one year. The plasma glucagon and somatostatin values following arginine infusion were very similar regardless of blood glucose control. The plasma glucagon response to arginine was lower and plasma somatostatin response higher in long-term diabetic patients without residual B-cell function than in normal subjects. The degree of blood glucose control has little influence on plasma glucagon and plasma somatostatin in long-term diabetes. Long-term Type 1 diabetic patients may have an intrinsic defect in the A-cell.

208. Two-year prospective study of improved control in diabetic patients with background retinopathy

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To assess the effect of gradually improved control on established diabetic retinopathy, 28 poorly controlled diabetic patients ($HbA_1 > 10\%$) on insulin or oral agents, who had not experienced home blood glucose monitoring, were studied. One eye of each patient was observed. Each eye had a visual acuity of 6/9 or better, no previous laser treatment, background retinopathy and < grade 1 disc new vessels. Diabetic control was improved by blood glucose monitoring combined with monthly consultations with a specialist nurse. The mean HbA_1 diminished from 12.1% to 9.6% over 6 months and over the next 18 months the mean value was 8.8%. The mean micro-aneurysm count assessed by fluorescein angiography increased over the first 6 months and then decreased to less than the initial count after 2 years. There was a strong negative correlation with the change in HbA_1 ($p<0.01$). Goldman perimetry showed improvement in macular function ($p<0.05$) but constriction of the peripheral field ($p<0.05$). Visual acuity, colour vision and grading of fundus photographs showed no significant correlation with HbA_1 or time. These results suggest that even a slow improvement in diabetic control leads to an initial deterioration in retinal morphology but that subsequently a gradual improvement may occur.

209. Metabolic and biochemical studies on human islets of Langerhans

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Very few biochemical studies on isolated human islets of Langerhans have been performed. Such information is important in validifying the application of models for insulin secretion, obtained from studies on animal islets, to the human islet. In the present study, human islets were isolated by collagenase digestion from the pancreas of a kidney donor. Maintainence of the islets in tissue culture enabled insulin release, glucose oxidation and Ca^{2+}-calmodulin-dependent protein phosphorylation to be determined from the same islets. Increasing glucose over a range from 0 to 20 mmol/l resulted in a sigmoidal stimulation of insulin release (from 32.98 ± 6.84 to 190.60 ± 19.53 μU/islet per h ($n=5$); threshold = 4 mmol/l). There was a marked correlation ($r=0.93$) between the insulin secretory response of the islets to glucose and their rate of glucose oxidation (5.3 ± 0.2 pmol/islet per h at 2 mmol/l glucose up to 23.9 ± 2.4 pmol/islet per h at 20 mmol/l). Extracts of the islets contained a Ca^{2+}-calmodulin-dependent protein kinase which phosphorylated a 48 kdalton endogenous polypeptide. Myosin light chain kinase was demonstrated by addition of exogenous myosin light chains. This report demonstrates, for the first time, the sigmoidal nature of glucose-stimulated insulin from isolated human islets and its correlation with enhanced glucose oxidation. Furthermore, this is the first report of the presence of Ca^{2+}-dependent protein kinases in human islets.

210. Remission in newly-diagnosed Type 1 (insulin-dependent) diabetes after continuous subcutaneous insulin infusion: frequency, duration and prediction factors

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The frequency of remissions after intensive insulin treatment in newly-diagnosed Type 1 diabetic patients has to be accurately assessed before immunosuppressive drugs can be used. In this study, we show that a 58% remission rate is obtainable by continuous subcutaneous insulin infusion (CSII) and that a decreased ratio of OKT4/OKT8 has a negative predictive value. Fifty patients (mean age 29 ± 13 years) were all newly diagnosed as insulin dependent by the following criteria: (1) recent weight loss, (2) massive ketonuria, (3) low molar ratio of C-peptide reactivity versus fasting glycaemia ($22.3 (\pm 13.5) \times 10^{-9}$). Islet cell antibodies were present in 70% and DR3 or DR4 in 76%. After 10 days of CSII (mean glycaemia 6.77 ± 1.21 mmol/l), patients were intentionally withdrawn from insulin treatment. In 58% (28 out of 50), remission occurred as defined by (1) satisfactory glycaemia without insulin treatment (fasting glycaemia $\leqslant$ 6.4 mmol/l, post-prandial $\leqslant 8$ mmol/l, glycosylated haemoglobinaemia $\leqslant$ normal value ± 2 SD), and (2) a duration of more than 3 months with stable body weight with or without oral hypoglycaemia drugs (glibenclamide and/or metformin). Mean duration of remission was 16 months (range: 3–34 months). Frequency of remission was higher when CSII (1) was started soon after clinical onset ($p \leqslant 0.02$), (2) achieved euglycaemia ($p<0.029$) and (3) utilized human insulin (Actrapid HM) (67% remission versus 46% with purified porcine insulin, Actrapid MC). The following factors have negative predictive value: weight loss ($p<0.001$), low insulinaemia ($p<0.001$), decreased ratio OKT4/OKT8 ($p \leqslant 0.015$), advanced age ($p<0.05$) and elevated triglyceridaemia ($p<0.05$).

211. Activated T cells in Type I (insulin-dependent) diabetes

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Islet cell antibody (ICA) and activated T cells bearing Ia (DR) antigens are detectable in the peripheral blood of diabetic patients prior to the onset of Type I diabetes. We have studied T cell subsets and Ia antigens, transferrin, T cell growth factor (TCGF) and insulin receptors on T cells in Type I diabetic patients. T cells express these receptors only upon activation. Peripheral lymphocytes were prepared from eight diabetic patients with positive ICA (mean age: 22 years) and four normal age-matched subjects. T cells were purified by passage through a nylon wool column followed by Petri dish culture. Ortho-Spectrum III was used for cytofluorographic analysis. There was no significant difference in the number of lymphocytes, OKT3-, OKT4-, and OKT8-T cells and the ratio of OKT4/OKT8 between the diabetic and control subjects. Ia antigens were detectable in seven of eight diabetic patient (87%), transferrin receptors (OKT9) in two out

eight, and TCGF receptors in two out of eight. Half of the diabetic patient showed I^{125}-insulin binding. No normal subjects showed these receptors. Activated T cells may play an important role in the pathogenesis of Type I diabetes.

212. Adipogenic activity in human serum

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The adipose conversion of 3T3 L1 fibroblasts, an established cell line, occurs only in the presence of serum in the incubation medium. Addition of human serum results in a dose-dependent increase of the key lipogenic enzyme glycerol-3-phosphate dehydrogenase. It is still unknown which serum components are responsible for the induction of the differentiation process. Of the growth-promoting substances investigated only growth hormone (GH) stimulated the adipose conversion of the cells. The half-maximally effective concentration of GH was found to be 5×10^{-11} mol/l, the maximal effect at 10^{-10} to 10^{-9} mol/l. A defined serum extraction procedure including heat treatment and ethanolic precipitation eliminated GH completely as assessed by different methods, Up to 50% of the original activity was preserved in the extract with a 22-fold increase of specific activity. Further purification was achieved by gel filtration and subsequent ion-exchange chromatography, suggesting a peptide-like substance with an apparent molecular weight of 4000–6000 daltons. The highest adipogenic activity was found in serum samples of neonates, twofold higher than in adults. No significant differences were detected between sera of lean and obese subjects and patients with non-insulin-dependent diabetes.

213. Insulin compatibility with polymer materials used in external pump infusion systems

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In the development of systems for drug infusion, various polymer materials are used for drug reservoirs and catheters. Compatibility of neutral insulin solutions with such polymer materials have been studied by examination of the physical and chemical stability of the insulin during contact with these products. Changes in pH, preservative concentration and insulin precipitation were monitored. Chemical transformation of insulin was analyzed by a 2-dimensional high performance liquid chromatography method estimating covalent di- and polymerization of the insulin as well as formation of other insulin derivatives. All polymer materials tested had a negative but varying influence on the quality of the insulin preparation. A heavy loss of preservative was observed in all catheters (*m*-cresol > phenol >> methylparaben). Covalent transformation of insulin was increased 5–40 times during contact with catheter materials. These chemical transformations take place to the same extent irrespective of the species and brand of insulin, but vary with the formulation of the insulin solution and type and treatment of catheter material. The major problem concerning insulin compatibility with polymer materials thus seems to be the chemical stability rather than the physical stability of insulin. None of the 10 different catheters tested showed ideal properties regarding insulin compatibility.

214. Defective suppression of endogenous proteolysis during meal absorption in Type I (insulin-dependent) diabetes corrected by insulin

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Insulin decreases the rate of appearance of leucine from body protein in post-absorptive man. To determine insulin's effect during meal absorption, seven insulin withdrawn (−INS) diabetic patients, five diabetic patients after intensive therapy (+INS) and five subjects were studied before and for 8 h after ingesting a chemically defined meal (10 kcal/kg), containing [1-^{14}C]leucine. Whole body leucine rate of appearance was determined with [$^2H_3$]leucine infusion. Baseline leucine flux was not significantly different among all three groups. During meal absorption total leucine rate of appearance was greater ($p < 0.01$) in the −INS diabetic patients than in normal or +INS subjects (1.4 ± 0.1 versus 1.0 ± 0.1 or 1.1 ± 0.1 mmol/kg for 8 h). Oxidation and entry of meal related leucine was similar in all three groups. During meal absorption, leucine rate of appearance from body protein was higher ($p < 0.01$) in the −INS patients than normal subjects (1.0 ± 0.1 versus 0.6 ± 0.1 mmol/kg for 8 h), but returned to normal with insulin (0.7 ± 0.1). In summary, in Type 1 diabetes (1) absorption and oxidation of dietary leucine was normal regardless of insulin therapy; (2) after meal ingestion, the rate of release of leucine from body protein was not suppressed in the absence of insulin, but this abnormality was corrected with insulin. In conclusion, insulin-mediated suppression of endogenous proteolysis is an integral part of protein anabolism during meal absorption.

215. Insulin infusion normalizes abnormal cardiovascular function and plasma noradrenaline after oral glucose in Tpye 1 (insulin-dependent) diabetes

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We have previously shown that intravenous injection of insulin increased plasma noradrenaline (NA), heart rate and the systolic blood pressure. Furthermore, an oral glucose load increased plasma NA, heart rate and blood pressure significantly in young normal subjects but not in young Type 1 diabetic patients without complications. The aim of this study was to examine if the abnormal regulation of the cardiovascular system and plasma NA observed after oral glucose in Type 1 diabetic patients was normalized by intravenous insulin infusion. Eight Type 1 diabetic patients were examined twice after an oral glucose load with and without intravenous infusion of insulin. Insulin infusion increased plasma insulin from 0.05 to 0.31 nmol/l. In the control experiment where only glucose was given, the heart rate and arterial blood pressure remained unchanged, whereas plasma NA decreased slightly. After oral glucose plus intravenous insulin the heart rate increased 11% ($p < 0.01$) and the systolic blood pressure increased 5% ($p < 0.01$). The plasma NA averaged 99.8% of basal values during the glucose load alone and 129% after glucose plus insulin ($p < 0.05$). Plasma adrenaline did not change. This study indicates that endogenous insulin participates in the regulation of the cardiovascular system and plasma NA during glucose intake.

216. Effects of mixing regular and intermediate acting insulins (NPH and Lente) on post-prandial blood glucose and plasma insulin levels in Type 1 (insulin-dependent) diabetes

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The effects of mixing short and intermediate acting insulins (NPH or lente) in the syringe, immediately before injection, on post-prandial blood glucose and plasma free insulin levels were investigated in 13 Type 1 diabetic patients. All were studied twice after an overnight fast. In a random order, Actrapid + Monotard (A + M) or Velosulin + Insulatard (V + I), U 40, semi-synthetic human insulin, was administered subcutaneously in the dosage: 12.7 U (range 4-36 U) short-acting and 32.2 U (16-52 U) intermediate-acting insulin. After 30 min, a standard liquid meal (500 Cal, 55% CHO) was taken over 15 min. Blood samples were taken every 15 min for 240 min. Mean ± SEM fasting and peak blood glucose levels for A + M and V + I were 9.9 ± 1.3 and 10.5 ± 1.2 mmol/l, and 15.7 ± 1.7 and 13.3 ± 1.5 mmol/l ($p < 0.02$), respectively. Post-prandial blood glucose levels after A + M were significantly higher from 60 to 240 min compared with V + I. At 240 min, blood glucose levels after A + M and V + I were 11.0 ± 1.7 and 6.4 ± 1.1 mmol/l ($p < 0.01$), respectively. Fasting and peak plasma free insulin levels for A + M and V + I were 20 ± 3 and 20 ± 3 mU/l, and 61 ± 7 and 94 ± 8 mU/l ($p < 0.001$), respectively. After V + I, plasma free insulin levels were significantly higher, compared with A + M, from 60 to 165 min. In conclusion, mixing regular and lente insulin in the syringe results in slower insulin absorption and higher post-prandial blood glucose levels than seen after administration of regular and NPH insulin mixtures.

217. Short-term interruption of wearing the pump: substitution requirements of insulin

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To discontinue wearing the pump for short periods enhances the feasibility of continuous subcutaneous insulin infusion therapy (CSII). We studied, in seven C-peptide, negative diabetics, the requirements of injected insulin to cover the interruption of CSII for 1 h (from 07.30 to 08.30 h.) during the day-time (from 08.00 to 20.00 h) and overnight (from 21.00 to 08.00 h). During 1 h interruption, with no insulin replacement, glucose levels remained comparable to those during CSII. During day-time discontinuance, Lente insulin was injected (dose equal to missed basal infusion dose), and pre-meal boluses (Actrapid) were given as during CSII. Glucose levels remained similar to CSII until 14.00 h, but rose thereafter to be twofold higher by 21.00 h during the substitution (14.2 ± 1.3 mmol/l) than CSII therapy ($6.9 \pm$

0.9 mmol/l, $p < 0.02$). Simultaneously, free insulin levels were lower during replacement than CSII therapy (5.0 ± 1.5 versus 12.9 ± 1.9 mU/l respectively, $p < 0.02$). During overnight interruption, 1.5 times the missed basal dose was injected as Lente at 21.00 h. Control remained comparable until 04.00 h. Thereafter by 08.00 h, plasma insulin declined and glucose rose more during replacement than CSII therapy (1.5 ± 0.3 versus 6.0 ± 1.2 mU/l and 12.2 ± 1.2 versus 6.1 ± 0.9 mmol/l, respectively, $p < 0.02$). In conclusion, (1) interruption of CSII for 1 h requires no insulin replacement; (2) 100% substitution for daytime and 150% for overnight maintains good control until the last few hours of discontinuance, when plasma insulin begins to decline.

218. Liberalized diet for Type 1 (insulin-dependent) diabetic patients on intensified insulin therapy: A long-term study

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Fourteen normal weight Type 1 diabetic patients (mean age 32 years, duration of diabetes 14 years) were selected from our diabetic clinic. During 1981–1982, they participated in our 5-day in-patient diabetes treatment and teaching programme which includes multiple daily use of short-acting and intermediary-acting insulin, regular blood glucose self-monitoring, self-adjustment of insulin dosages and a high carbohydrate mixed diet. Recording of fat, protein and caloric intake or the use of exchange lists of carbohydrates according to groups were not recommended. One year after the using the teaching programme, the patients eating habits were assessed over 5 consecutive days. The caloric intake was 1064–3460 Kcal/day (seven women) and 2020–6120 Kcal/day (seven men) with an individual day-to-day variability of 16% (mean variation coefficient MVC); distribution of nutritients: carbohydrates $39 \pm 9\%$ (MVC 18%), protein $16 \pm 5\%$ (MVC 27%), fat $41 \pm 9\%$ (MCV 24%). All patients performed blood-glucose monitoring two to five times daily with frequent insulin dose adjustments. The insulin dose was 51 ± 14 U/day, 41% being rapid-acting insulin. Body mass index increased from 21.4 ± 2.6 to 23.4 ± 1.2 (one year) to 23.5 ± 1.3 (2 years after participating in the teaching programme), HbA_{1c} fell from $9.3 \pm 1.7\%$ (before) to $7.8 \pm 0.7\%$ (after 2 years); serum lipids remained normal. Selected, well-trained, normal-weight Type-1 diabetic patients on intensified insulin therapy do not necessarily need rigid dietary prescriptions for fat, protein and caloric intake or carbohydrate exchange lists in order to achieve optimal metabolic control.

219. Effects of prolonged glucagon deficiency on ketogenesis in the 7-day fasted dog

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In 7-day fasted dogs somatostatin was infused with intraportal insulin ± glucagon for 3 h after a 40-min control period. Glucose was infused to maintain euglycaemia. Control hormone levels were unchanged (insulin 7 ± 1 mU/l, glucagon 42 ± 5 pg/ml) with combined infusion. Non-esterified fatty acide (NEFA), total ketone bodies (TKB) and their production (TKBP) fell (1.54 ± 0.27 to 0.85 ± 35 mmol/l, 253 ± 45 to 123 ± 43 µmol/l and 8.78 ± 1.51 to 2.16 ± 50 µmol $kg^{-1} \cdot min^{-1}$, respectively). When glucagon was not replaced insulin was unchanged (7 ± 1 mU/l) and glucagon, NEFA, TKB and TKBP fell: (59 ± 4 to 37 ± 6 pg/ml, 1.66 ± 0.03 to 0.64 ± 0.09 mmol/l, 318 ± 45 to 113 ± 13 µmol/l and 7.86 ± 1.36 to 1.34 ± 0.68 µmol $\cdot kg^{-1} \cdot min^{-1}$). To assess the role of glucagon during insulin deficiency these protocols were repeated with lower insulin infusion rates (57% decrease). Insulin declined (9 ± 1 to 6 ± 1 mU/l) and glucagon was unchanged (84 ± 14 pg/ml) with combined infusion. NEFA, TKB and TKBP fell slightly (0.98 ± 0.14 to 0.67 ± 0.17 mmol/l, 335 ± 43 to 229 ± 62 µmol/l and 9.73 ± 1.75 to 6.54 ± 1.19 µmol $\cdot kg^{-1} \cdot min^{-1}$). When glucagon was not replaced, insulin (12 ± 2 to 7 ± 1 mU/l), glucagon (57 ± 2 to 18 ± 7 pg/ml) NEFA (1.27 ± 0.49 to 0.77 ± 0.14 mmol/l), TKB (233 ± 37 to 182 ± 37 µmol/l), and TKBP (8.77 ± 2.80 to 3.53 ± 1.23 µmol $\cdot kg^{-1} \cdot min^{-1}$) fell. In conclusion, in 7-day fasted dogs prolonged glucagon deficiency does not affect ketogenesis while somatostatin in this case, unlike in 18 h fasted dogs, directly or indirectly inhibits lipolysis and TKBP.

220. Mechanisms of magnesium efflux from islet cells: analogies with and differences from calcium efflux

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Although it was recently shown that glucose modulates Mg efflux from pancreatic islets, the mechanisms regulating this efflux are unknown. Rat islets were loaded with ^{28}Mg ($t_{½} = 21$ h) and the efflux of the tracer was continuously monitored in a perifusion system. At low glucose (3 mmol/l), the rate of ^{28}Mg efflux decreased on omission of extracellular Mg and increased on addition of Mg to the medium. Substitution of choline for Na caused a large increase in ^{28}Mg efflux if extracellular Mg was present. This acceleration is due to facilitation of Mg influx. By contrast, omission of Na from a Mg-free medium inhibited ^{28}Mg efflux. A similar inhibition also occurred when the intracellular Na concentration was raised by blockade of the sodium pump (ouabain or K-free medium). In the absence of glucose, dibutyryl-cyclic AMP, isobutylmethyl-xanthine or forskolin slightly decreased the rate of ^{28}Mg efflux whether extracellular Ca or Mg was present or not. Under these conditions, they increased ^{45}Ca efflux. These results suggest that, in pancreatic islet cells, Mg efflux is activated by extracellular Mg and Na, possibly through Mg/Mg and Na/Mg exchanges (analogous to the Ca/Ca and Na/Ca exchanges) and that cyclic AMP may exert opposite effects on Ca and Mg efflux.

221. Forskolin, an activator of adenylate cyclase, and the endocrine pancreas

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Forskolin, a direct activator of adenylate cyclase, stimulates cyclic AMP production in islet cells. The effects of forskolin on the release of somatostatin, glucagon and insulin were studied using the isolated, perfused dog pancreas. It was found (1) that forskolin at concentrations ranging from 0.075 – 1.00 µmol/l stimulated the secretion of somatostatin, glucagon and insulin in a dose-related manner. (2) The effects of forskolin (0.15 and 0.6 µmol/l) were modulated by the prevailing glucose level with higher D- and B- and lower A-cell responses at high (9.7 mmol/l) rather than low glucose (2.8 mmol/l). (3) In the absence of extracellular Ca^{++}, forskolin (0.6 µmol/l) possessed no stimulatory effect on pancreatic hormone secretion. (4) The phosphodiesterase inhibitor 3-isobutyl-l-methyl-xanthine (IBMX) (25 µmol/l) elicited qualitatively similar hormone responses to forskolin. (5) Perfusing atropine (1 µmol/l), propranolol (1 µmol/l) and phentolamine (1 µmol/l) had no effect on forskolin-mediated (0.3 µmol/l) hormone output from pancreas. In conclusion, the experiments demonstrate that forskolin is a potent, reversible, stimulus of pancreatic hormone secretion. Its effects are apparently not mediated via the sympathetic or parasympathetic nerve endings in pancreas. Forskolin may prove to be a valuable pharmacological tool in probing the role of the adenylate cyclase cyclic AMP system in pancreatic hormone secretion.

222. Effects of epicatechin and quercetin on $^{45}Ca^{2+}$ handling by isolated rat islets of Langerhans

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We have previously demonstrated that epicatechin, a flavonoid, increased both insulin secretion and total cyclic AMP content in isolated islets. Recent work suggested that another flavonoid, quercetin, is more potent than epicatechin in enhancing insulin secretion. The effects of these flavonoids on the uptake and efflux of $^{45}Ca^{2++}$ were investigated. In uptake studies, islets were double-labelled with $^{45}Ca^{2+}$ and (^{3}H)-sucrose (5 min) in medium containing 2 or 20 mmol/l glucose, followed by centrifugation through oil. $^{45}Ca^{2+}$ efflux was investigated by perifusing prelabelled islets in the presence or absence of extracellular Ca^{2+}. At 2 mmol/l glucose, the flavonoids did not significantly increase $^{45}Ca^{2+}$ uptake; insulin secretion was enhanced only by epicatechin (0.8 mmol/l). At 20 mmol/l glucose, both flavonoids increased insulin secretion ($p < 0.05$); $^{45}Ca^{2+}$ uptake was enhanced by 27.1% by epicatechin ($p < 0.01$) and 35.8% by 10 µmol/l quercetin ($p < 0.005$). The flavonoids inhibited the increased $^{45}Ca^{2+}$ efflux brought about by 20 mmol/l glucose in the presence of extracellular Ca^{2+} (epicatechin: $p < 0.05$; quercetin: $p < 0.002$). In Ca^{2+} free medium, basal efflux at 2 mmol/l glucose was also inhibited by both flavonoids ($p < 0.002$). These results suggest that epicatechin and quercetin may, in part, exert their effects on insulin secretion via an increase in Ca^{2+} accumulation in β cells.

223. Subcutaneous absorption of human and bovine ultratard insulins

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The subcutaneous absorption of ^{125}I-labelled semi-synthetic human insulin (Ultratard HM) was compared with that of ^{125}I-labelled beef insulin (Ultratard MC; also named Ultralente MC) in eight insulin-

dependent diabetic patients according to a balanced cross-over design. In a randomized way, 6 IU of Ultratard HM was given in the thigh and abdomen, whereas 24 IU Ultratard HM was given in the contralateral thigh and 6 IU Ultratard MC in the abdomen. On a later occasion, the injection sites of Ultratard HM and Ultratard MC were reversed. Ultratard HM was absorbed significantly faster than Ultratard MC. Thus, the time found for a 50% disappearance of the initial radioactivity on the thighs was 15 h versus 35 h for the high dose and 12 h versus 17 h for the small dose for Ultratard HM and Ultratard MC, respectively. The difference between the two doses was statistically significant for Ultratard MC. Ultratard HM, but not Ultratard MC, showed faster absorption from the abdomen than from the thigh. The results indicate that Ultratard HM is a human insulin preparation which has a prolonged action that could make it valuable as a basal insulin preparation used in multiple insulin injections regimens.

224. Effect of two new glucosidase inhibitors (BAY m 1099 and BAY o 1248) on post-prandial blood glucose and serum insulin levels in normal subjects

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Desoxynojirimycin derivatives (BAY m 1099 and BAY o 1248) are new potent glucosidase inhibitors. In animals, the post-prandial rise of blood glucose and serum insulin after sucrose loading or mixed meals was reduced by oral treatment with BAY m 1099 and BAY o 1248. We investigated the reproducibility of these findings in man. In seven cross-over studies, 42 healthy volunteers received three mixed meals: 900 cal each, 20% protein, 35% fat, 45% carbohydrate (30% sucrose). BAY m 1099 (25, 50, 100 or 200 mg) and BAY o 1248 (10, 20 or 40 mg) were given orally at the beginning of breakfast. Blood glucose and serum insulin levels were measured before and up to 3 h after each meal. The post-prandial rises of blood glucose and serum insulin were significantly dose-dependently decreased by BAY m 1099 for up to 3 h after administration. At the 100–200 mg doses flatulence and diarrhoea were reported. BAY o 1248 decreased the post-prandial blood glucose and serum insulin rise dose-dependently after breakfast, lunch and dinner. Intestinal symptoms (flatulence, diarrhoea) were dose-dependent. Based on these findings, we conclude that the new glucosidase inhibitors (BAY m 1099 and BAY o 1248), which are respectively short- and long-acting, are well tolerated and clinical studies in subjects with diabetes are well justified.

225. Electrocardiographic abnormalities in non-insulin-dependent diabetic patients at diagnosis

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Resting electrocardiograms (ECG) were performed on 247 newly diagnosed non-insulin-dependent diabetic patients under 66 years of age before starting hypoglycaemic treatment. 39 patients (16%) had substantial abnormalities (assessed by Minnesota coding), namely Q-waves in 4% of all ECGs, ST depression in 4%, T-wave inversion in 7%, conduction defects in 3% and dysrhythmias in 4%. Compared with the other 208 patients, these 39 were older (56 versus 50 years, $p=0.002$), had higher systolic blood pressure ($p=0.04$) and greater heart size by cardio-thoracic ratio ($p=0.01$). Fasting plasma insulin was higher in those with abnormalites (12.4 versus 10.9 mU/l, $p=0.03$), particularly when correlation of body mass index with plasma insulin was allowed for ($p=0.01$) (the two groups did not differ in mean body mass index). The area under the insulin secretory curve for 90 min after intravenous injection of 20 g glucose/m^2 body surface was also increased in those with abnormal ECG ($p=0.03$). Inclusion or exclusion of patients already on diuretics and/or hypotensive agents did not alter the general results. The groups did not differ significantly in fasting plasma glucose or cholesterol concentrations, nor their histories of tobacco or ethanol consumption.

226. Increased β-adrenergic sensitivity in diabetic autonomic neuropathy

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Adrenaline (6 μg/min) was infused in diabetic patients without neuropathy (group 1; $n=7$), diabetic patients with autonomic neuropathy (decreased beat-to-beat variation and orthostatic hypotension; group 2, $n=7$) and in normal subjects (group 3, $n=7$). Age and duration of diabetes were similar in all groups. The study was performed after an overnight insulin infusion, aiming at normoglycaemia. Plasma catecholamines were similar in all groups during the experiment, and free insulin levels were similar in groups 1 and 2. Heart rate increase after adrenaline was greater in group 2 compared with groups 1 and 3 [mean ± SEM: 76 ± 4 (versus 108 ± 3 beats/min group 2), 64 ± 3 versus 81 ± 3 (group 1) and 59 ± 3 versus 74 ± 2 (group 3; $p<0.01$]. Mean arterial blood pressure was unaffected in groups 1 and 3, whereas a significant decrease was found in group 2 (105 ± 3 versus 83 ± 3 mmHg, $p<0.01$). The increase in cardiac output was similar in all groups, whereas the percentage decrease in total peripheral vascular resistance was significantly greater in group 2 compared with groups 1 and 3 [59 ± 5% (group 2), 34 ± 6% (group 1) and 41 ± 6% (group 3); $p<0.05$]. Blood glucose increase was significantly enhanced in group 2 compared with groups 1 and 3 [5.6 ± 0.5 versus 13.5 ± 0.8 mmol/l (group 2), 6.0 ± 1.0 versus 11.4 ± 1.1 (group 1) and 4.9 ± 0.2 versus 7.1 ± 0.6 (group 3); $p<0.05$]. Thus, adrenaline induces an exaggerated fall in peripheral vascular resistance and an enhanced blood glucose increase in diabetic patients with autonomic neuropathy. These findings are compatible with increased β-adrenergic sensitivity in diabetic autonomic neuropathy.

227. Is there linkage of DNA inserts near the insulin gene with Type 1 (insulin-dependent) diabetes? – a pedigree analysis

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Recently Type 1 diabetes has been shown to associate with patients homozygous for the class 1 DNA insertion adjacent to the insulin gene (localised to chromosome 11). Using pedigree analysis, we have sought for linkage of class 1 inserts with Type 1 diabetes. 14 pedigrees were studied (each having two or more diabetic subjects), which were subdivided into multiple generation (MG) or multiple sibling (MS) affected families. DNA was prepared from leucocytes of 29 diabetic patients and 55 non-diabetic subjects, digested with restriction enzymes, Southern-blotted and hybridised with a ^{32}P-labelled insulin gene probe. Two major gene related fragments were observed by autoradiography containing either small (class 1) or large (class 3) polymorphic DNA insertions adjacent to the insulin gene. Linkage was computed for each family by Lod scores using an autosomal dominant or an autosomal recessive model with 50% penetrance for the MG and MS families respectively. Four families (all MS) were informative, the total Lod score being −3.7 (for a recombination fraction of 0). In conclusion, no close linkage of the insulin gene polymorphism was found with Type 1 diabetes in the multiple sibling affected families.

228. Increased non-insulin stimulated glucose transport and improved insulin responsiveness of glucose transport in fat cells after conventional treatment of Type 2 (non-insulin-dependent) diabetic patients for one year

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Eight Type 2 diabetic patients were studied at the time of diagnosis and again 1 year after starting treatment. Seven patients had been treated with diet alone, one patient with diet and glibenclamide. Fasting plasma glucose decreased from 11 ± 1.4 to 7 ± 0.9 mmol/l ($p<0.01$). Fasting serum insulin was unchanged. At the time of second examination, no patients had glycosuria. Weight loss was 7 ± 7 kg. Adipocyte insulin binding at tracer concentration (15 pmol/l, 37 °C) increased from 1.64 ± 0.54 to 2.05 ± 0.72% (30 cm^2 surface area/ml, $0.1>p>0.05$). The basal (non-insulin-stimulated) glucose transport rate in fat cells (D-U-^{14}-C-glucose concentration 5 μmol/l) increased from 25.6 ± 12.9 to 43.2 ± 14.9 pmol/90 min per 10 cm^2 surface area ($p<0.02$). The maximal insulin-stimulated glucose transport rate increased from 34.9 ± 20.6 to 76.9 ± 27.4 pmol/90 min ($p<0.01$). The percentage insulin response above basal levels increased from 42 ± 41 to 87 ± 58% ($p<0.01$). The increase in percentage insulin response was correlated to weight loss ($r=0.86$, $p<0.05$). No change in half maximal stimulating insulin concentrations was found. Glucose conversion rate to total lipids increased 34 ± 64% and 65 ± 81% in basal and maximally insulin stimulated cells respectively (NS). The percentage insulin response above basal level increased from 29 ± 29 to 66 ± 53% ($p=0.06$). In conclusion, conventional treatment of Type 2 diabetes improves basal glucose uptake and effectiveness of insulin on glucose uptake and metabolism in fat cells.

229. Enhancement of antigen-antibody reaction by protamine in vitro

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Protamine is a component of many 'retard' insulin preparations. Beside the retardation of insulin absorption, it has various biological effects. We developed a laser-nephelometric equivalence point titration technique to study the effect of drugs on the degree of lattice formation of specific antigen-antibody complexes in vitro. In this system, serial dilutions of the antigen incubated with a constant amount of antibody and the influence of the added drug on complex formation in antigen excess, at equivalence, and in antibody excess were measured as light scatter intensity. We found that protamine sulphate was a potent adjuvant which enhanced specific complex formation in antibody excess. It stabilized soluble complexes in antigen excess and provoked a shift of the equivalence point to higher antigen concentration comparable with the known adjuvant, polyethyleneglucol (PEG). In contrast to PEG, the effect of protamine depended on the ionic strength of the solvent and could be eliminated by heparin. The adjuvant effect of protamine was clearly distinguishable from the non-specific aggregation of immunoglobulins by protamine and was found in all systems examined (albumin-anti-albumin, Ig + anti-Ig etc). This could be of clinical relevance by an influence on the kinetics of specific insulin-insulin-antibody reactions in vivo.

230. Glucose and fluid transport across the small intestine in the streptozotocin-diabetic rat

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Streptozotocin-diabetes in the rat is accompanied by weight loss and paradoxical hyperplasia of small intestinal mucosa, unexplained by hyperphagia and associated with increased DNA synthesis and enhanced sugar transport. In this study the effect of streptozotocin diabetes on glucose transport and metabolism, and fluid transport across rat jejunum was investigated in vitro. The lumen of an isolated segment of jejunum was recirculated with a medium containing glucose (28 mmol/l), and serosal fluid samples collected over 100 min. Mild streptozotocin-diabetic rats after 1, 3 and 5 weeks were compared with age-matched controls. Control rats gained 170 g in weight during 5 weeks, with no change in small intestinal length, whilst the diabetic rats gained no weight but the small intestinal length increased significantly by 30 cm (25%). In the diabetic rats fluid transport rate increased significantly ($p < 0.01$) between weeks 1 and 3 from 0.32 ± 0.01 to 0.46 ± 0.03 ml/g dry weight per min, while the rate of glucose transport increased significantly ($p < 0.01$) from 12.3 ± 0.3 to 18.6 ± 1.3 μmol/g dry weight per min, both remaining significantly higher than in control rats thereafter. Serosal lactate appearance was similar in all groups. It is concluded that in streptozotocin-diabetic rat jejunum there is increased glucose transport with unaltered metabolism to lactate, and increased fluid transport which may significantly assist fluid replacement in diabetes.

231. Lack of benefit from guar administration in well controlled non-insulin-dependent diabetic patients

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Non-insulin-dependent diabetic patients with near-normal fasting plasma glucose levels often have moderately elevated HbA_1, levels probably reflecting their excessive post-prandial glycaemia. 29 non-insulin-dependent diabetic patients (24 males, 5 females, mean ± SD age 54.2 ± 10.7 years, body mass index 26.5 ± 3.1 kg ÷ m^2 with fasting plasma glucose < 7 mmol/l consented to a randomised cross-over study. Eleven were treated by diet alone, eight with a sulphonylurea and ten with once daily ultralente insulin. Patients were given guar granules (5 g sachet three times daily, MCP Pharmaceuticals) or placebo (one tablet three times daily) each for an 8 week period before a standardised inpatient 24 h metabolic profile. No differences were apparent between guar and placebo therapy in post-prandial glycaemic excursion (plasma glucose 1 h after breakfast 9.8 ± 2.4 versus 9.5 ± 2.2 mmol/l), mean 24 h plasma glucose levels (6.3 ± 1.3 versus 6.4 ± 1.6 mmol/l), HbA_1, values (8.1 ± 2.0 versus 7.8 ± 1.8%) or body weight (77.6 ± 9.6 versus 77.6 ± 9.2 kg). Reported side effects were not different and infrequent with either therapy. In conclusion, the benefit of guar additives in improving diabetic control has yet to be proven in everyday clinical use.

232. Loss of normal sigmoid, beta-cell dose-response curve to glucose in non-insulin-dependent diabetes

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Five non-insulin-dependent diabetic subjects (NIDD) on diet only (fasting plasma glucose 5.5–6.4 mmol/l) and seven non-diabetic subjects (fasting blood glucose 4.6–5.5 mmol/l) were studied using hyperglycaemic clamp. Each subject was clamped at three different levels of hyperglycaemia on separate days (7.5, 10 and 15 mmol/l) for 2.5 h. Plasma C-peptide levels over last 30 min of each clamp were used to assess pre-hepatic insulin production. All normal subjects had a sigmoid glucose-C-peptide dose-response curve with the steepest response between glucose levels of 7.5 and 10 mmol/l, whereas NIDD had a near linear dose-response curve, with a lower gradient than the normal subjects over the 7.5–10 mmol/l range. Between glucose levels of 10 and 15 mmol/l normal subjects had a flatter response (increase of 22 ± 29%) than NIDD (increase 146 ± 78%, $p < 0.01$). Basal C-peptide values were similar (mean ± SD, normals subjects 0.46 ± 0.21, NIDD 0.49 ± 0.21 pmol/ml) as were 7.5 mmol/l clamp values (1.28 ± 0.7 and 0.9 ± 0.21 pmol/ml, respectively) whereas NIDD had reduced levels at 10 mmol/l (normal subjects 3.72 ± 1.87 NIDD 1.15 ± 0.41 pmol/ml, $p < 0.01$) but similar levels at 15 mmol/l (normal subjects 4.16 ± 1.58 NIDD 2.74 ± 1.18, NS). In conclusion, dose-response curves in NIDD show β-cell insensitivity at physiologically important levels of hyperglycaemia, rather than simple reduction of maximal response.

233. Islet cell antibodies: a non-specific marker for the development of insulin-dependent diabetes

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The identical twin of a newly diagnosed insulin-dependent diabetic carries an approximately 60% risk of developing the disease. The presence of islet cell antibodies (ICA) might indicate which twins will develop diabetes. Of 17 non-diabetic co-twins tested for ICA 5 or more years ago, nine were initially positive (seven with complement-fixing antibodies), of whom six have since become negative. All 17 twins continue to have normal glucose tolerance. We can calculate the cumulative risk of these twins developing diabetes from the concordance rates in our larger twin series. All nine antibody-positive twins have been discordant for > 7 years, after which time the risk of unaffected twins developing diabetes is < 30% and four have been discordant for > 13 years, when the subsequent risk of developing diabetes is < 3%. Thus, it is highly probable that ICA in genetically susceptible subjects do not always presage insulin-dependent diabetes.

234. Hexokinase isoenzymic distribution in rat sciatic nerve and spinal cord: effects of experimental diabetes

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Hexokinase (HK) regulates the entry of glucose into the various pathways of carbohydrate metabolism. HK isoenzyme distribution within tissues is variable; a high proportion of type II is characteristic of insulin sensitive tissues, whilst type I is associated with insulin insensitivity. HK distribution between the cytosol and mitochondrial membrane is variable. Binding is controlled by cellular levels of ATP, glucose-6-phosphate (G-6-P) and Pi and, when bound, exhibits a decreased susceptability to G-6-P inhibition and an increased affinity for ATP. When HK was measured in fractionated extracts from sciatic nerve, predominantly (80%) type II was found, with an equal distribution between cytosol and G-6-P eluted particulate fractions. In the spinal cord mainly type I (65%) was found, with the remainder being type III. The subcellular distribution is similar to brain with 70% HK bound. Experimental diabetes caused a decrease (30–50%) in sciatic nerve HK, this effect was present in both compartments and in both short- (2–3 weeks) and long- (9 months) term diabetes. In spinal cord, at only 9 months was any change evident, a 30% increase in both compartments. These results are indicative of an insulin-sensitivity in sciatic nerve with a resultant "under" utilization of glucose contrasting with spinal cord where glucose "over" utilization is manifested.

235. Stimulatory effect of vasopressin, angiotensin II and glucagon on gluconeogenesis and ketogenesis in the perfused rat liver

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The stimulatory effect of glucagon on gluconeogenesis and ketogenesis in the perfused rat liver is well established. It is not yet clearly understood, however, whether or not vasopressin and angiotensin II enhance hepatic gluconeogenesis and ketogenesis. Therefore, these two hormones were perfused in the presence of 10 mmol/l alanine for 2 h

in isolated liver from 24-h-starved normal rats. Both vasopressin (100 nmol/l) and angiotensin II (0.8 U/l) caused a 150–230% increase in hepatic glucose production ($p < 0.01$), following a decrease of lactate ($p < 0.05$). Ketone body production also was significantly increased by the two hormones (control: 23.1 ± 2.5 μmol/g liver, vasopressin: 65.0 ± 7.0, angiotensin II: 66.7 ± 10.0, $p < 0.01$). This increase was observed in β-hydroxybutyrate ($p < 0.01$) and acetoacetate ($p < 0.05$). Ureogenesis was not affected by these hormones. Insulin (100 mU/l) suppressed gluconeogenesis by 60% with angiotensin II and by 50% with vasopressin ($p < 0.01$). However, there was little inhibitory effect of insulin on ketogenesis increased by angiotensin II and vasopressin. Similar results were observed also with glucagon in the livers from streptozotocin-diabetic rats.

236. Influence of lactation upon adenylate cyclase activity in pancreatic rat islets

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The insulin-secretory behaviour of islets removed from lactating rats differs from that of non-lactating animals. The possible involvement in such a behaviour of an adaptative change in adenylate cyclase activity was investigated. Virgin female rats were mated and, after delivery, divided into a lactating and a non-lactating group. In the latter group, pups were removed immediately after delivery. Pancreatic islets were isolated 14 days after delivery. Adenylate cyclase activity was measured both in a crude homogenate and a subcellular particulate fraction (20 min, 12000 *g*). Whether in the crude homogenate or subcellular particulate fraction, the basal activity was 20–30% higher in lactating than in non-lactating animals. GTP (10 μmol/l) augmented adenylate cyclase activity to the same relative extent in lactating and non-lactating animals. However, the activation of the enzyme by either NaF (10 mmol/l) or forskolin (10 μmol/l) was more pronounced in the lactating rats, even when expressed relative to the higher basal value found in these animals. As a result, the paired ratio in reaction velocity of lactating/non-lactating animals averaged 1.52 ± 0.15 and 1.85 ± 0.25 in the presence of forskolin and NaF respectively. These results indicate that lactation, like pregnancy, increases adenylate cyclase activity and sensitivity in the pancreatic islets.

237. Response to insulin deprivation in severe brittle diabetes

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To test the hypothesis that frequent ketoacidosis in severe brittle diabetes results from an exaggerated response to insulin deficiency, the metabolic response to insulin deprivation has been compared in 16 female brittle diabetic patients, and six age-, sex- and weight-matched C-peptide, negative stable diabetic patients. After 60 h treatment with short-acting insulin only, and overnight control with intravenous insulin, insulin was stopped and blood glucose, metabolites and hormones measured for 4 h. Blood glucose rose to higher levels in the brittle diabetics (22.8 versus 17.0 mmol/l, $p < 0.001$) but a similar rise in 3-hydroxybutyrate concentration was found. Concentrations of non-esterified fatty acids, free insulin, and counter-regulatory hormones were not different basally or after insulin deprivation. Concentrations of lactate, pyruvate, alanine and glycerol were significantly elevated basally in the brittle patients, but fell into the stable diabetic range after insulin withdrawal. Basal lactate and pyruvate correlated with the overnight insulin delivery rate (lactate r_s 0.62, $p < 0.05$; pyruvate r_s 0.70, $p < 0.05$). These previously noted abnormalities could thus be the result of therapeutic hyperinsulinisation of the brittle patients. Thus, although brittle diabetics show clear metabolic abnormalities and increased hyperglycaemia after insulin withdrawal, neither an exaggerated response to insulin deficiency, nor elevated levels of counter-regulatory hormones, can explain the frequent ketoacidosis in these patients.

238. A survey of the nutritional status of insulin-dependent diabetic patients in the west of Scotland

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In a cross-sectional survey, the weight, vitamin and trace mineral status of 57 insulin- and diet-controlled diabetic patients (27 males, 30 females, mean age 48.6 years; range 12–79 years) were studied. Twenty-six patients with HbA_1 levels of $\leq 12\%$ (group 1) were slightly heavier (mean ± SD percentage of ideal weight = 111.2 ± 12.6% compared with 107.8 ± 17.3%) and had slightly lower mean blood glucose levels (mean ± SD 11.5 ± 3.6 mmol/l compared with 12.9 ± 3.8 mmol/l) than the 31 patients with HbA_1 levels of $> 12\%$ (group 2). Clinical assessment of control by numerical score produced no significant difference between the groups. No individual patient was found to have evidence of gross nutritional deficiency. However, seven patients (six in group 2) were found to have serum magnesium levels below the normal range, and the overall mean ± SD serum magnesium was 0.75 ± 0.06 mmol/l (normal range 0.7–1.0 mmol/l). This may have clinical implications in the development of cardiac arrhythmias in patients who already have an increased risk of heart disease. We conclude that routine nutritional assessments are not necessary in insulin-dependent diabetic patients unless clinically indicated, but serum magnesium levels should be monitored regularly.

239. Phorbol ester stimulation of secretory granule phosphorylation and insulin release in a transplantable rat insulinoma

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The possible role of Ca^{2+}- and phospholipid-dependent protein kinases (protein kinase C) in insulin release was assessed from a study of the effects to tumour-promoting phorbol esters on protein phosphorylation reactions and secretion in rat insulinoma tissue. 4 β-phorbol 12-myristate 13-acetate (TPA) was a potent secretagogue at concentrations above 10^{-7} mol/l. TPA-induced release was inhibited by adrenaline or omission of Ca^{2+} from the extracellular medium and was augmented by theophylline. These findings suggested that TPA activated an exocytotic process. Insulinoma-soluble proteins catalysed the Ca^{2+}- and phospholipid-dependent phosphorylation of exogenous histone III-S and a number of endogenous substrates. The latter included soluble proteins and proteins associated with the insulin secretory granule membrane. TPA markedly enhanced these reactions both in the absence or presence of Ca^{2+}. Histone phosphorylation and the endogenous protein phosphorylation reactions involving the secretory granule showed similar concentration dependencies for activation by both Ca^{2+} and TPA. This suggested that the same enzyme(s) was involved. It is proposed that protein kinase C undergoes reversible Ca^{2+}-dependent association with intracellular membrane in the β cell upon elevation of the cytosolic Ca^{2+} concentration. Resultant phosphorylation reactions may be important in the regulation of insulin secretion.

240. Varying organization of gap junctional particles in pancreatic β-cell membranes

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Gap junctions (GJ) are thought to mediate communication between pancreatic β cells. Conditions which alter the number of GJ in islets have been associated with variations in β-cell function. This study examines whether the assembly of GJ subunits represents a regulatory site in this process. In rat islets in situ, 60% of the 9 nm membrane particles were polygonally packed in GJ, while the remaining part occurred in linear strands. After collagenase isolation, the islets contained similar numbers of GJ but virtually no linear strands. In cultured islets, the particle distribution over polygonal or linear arrays varied with the culture conditions: in the presence of dibutyryl-cyclic AMP, theophylline or 11.2 mmol/l glucose significantly more particles occurred in GJ than at 5.6 mmol/l glucose alone. Newly formed β-cell aggregates also contained GJ and linear particle arrays: addition of theophylline plus glucagon increased the cellular cyclic AMP content and the percentage of polygonally packed particles. These results suggest a dynamic nature of islet gap junctions. In long-term experiments, glucose or cyclic AMP induce the assembly of linear strand particles into the polygonal arrays of GJ. It is conceivable that intercellular communication is not only modulated via the formation of GJ-subunits, but also via their organization in the β-cell membrane.

241. Behaviour of impotence in Type I (insulin-dependent) diabetic patients after 12 months' improved metabolic control

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In a prospective, controlled, non-randomized trial the influence of improved metabolic control over a 12-month period on male impotence was observed. Two groups were examined. Pump-group: $n = 18$, age 30 ± 14 years duration diabetes 15 ± 6 years; external or implanted in-

sulin infusion device (intravenous or intraperitoneal). Control-group: $n = 18$, matched for age, duration of diabetes and daily alcohol and/or nicotine intake. All patients had a peripheral (motor and/or sensory) neuropathy. *Examination:* Questionnaire for sexual behaviour, autonomic symptoms, daily alcohol and nicotine intake; 30:15-ratio and Valsalva-ratio; motor (N. peroneus) and sensory (N. suralis) nerve conduction velocity. *Results:* For evaluations patients were divided into three sub-groups: N-N = no alcohol or nicotine intake; L-L = ⩽ 8 mg nicotine and ⩽ 10 g alcohol intake daily (min. 2 mg resp. 3 g); S-S = > 8 mg nicotine and > 10 g alcohol intake daily.

Months	Pump-group			Control-group		
	0		12	0		12
Mean blood glucose (mmol/l)	13.9	*	7.8	11.9	NS	12.0
HbA_1 (%)	12.3	*	8.2	11.6	NS	11.9
Patients with impotence:						
N-N ($n = 6$)	6	*	1	6	NS	6
L-L ($n = 6$)	6	NS	4	6	NS	5
S-S ($n = 6$)	6	NS	6	6	NS	6

* = $p < 0.005$

Near-normoglycaemic control over a 12-month period improved impotence in male diabetic patients only if they were non-smokers and took no alcohol.

242. Anti-diabetic activity of Ro 16-8714, a β-adrenergic agonist, in obese hyperglycaemic (ob/ob) mice and streptozotocin-diabetic rats

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Ro 16-8714, a phenethanolamine derivative, exhibits pronounced calorigenic activity mediated directly by β-adrenergic receptors. Prolonged treatment of obese animals produced significant reductions in body weight and body fat, independent of which potent anti-diabetic effects were observed in obese or streptozotocin-induced mice (STZ: 70 mg/kg subcutaneously). In daily treatment of obese mice for 15 days (60 μmol/kg) glycosuria fell by 68% during the first week and subsequently disappeared. Blood glucose was rendered normal while plasma insulin was essentially unchanged. In STZ-diabetic mice (6 $\mu mol \cdot kg^{-1} \cdot day^{-1}$ for 3 weeks followed by 60 $\mu mol \cdot kg^{-1} \cdot day^{-1}$ for 2 weeks) glycosuria was decreased by 40% during week 5 of treatment and hyperglycaemia was reduced to the same extent. Oxygen consumption was elevated by 22% in obese mice and 10% in STZ diabetic mice. Indirect calorimetry revealed a significant increase in carbohydrate oxidation. Ro 16-8714 caused a marked hypertrophy of interscapular brown adipose tissue, and in obese mice treated for 29 days a 6–7-fold increase in brown adipose tissue mitochondrial GDP-binding, indicating enhanced brown adipose tissue thermogenesis. In STZ-diabetic mice, GDP-binding increased 3.5-fold. The antidiabetic qualities of Ro 16-8714 may result from a marked increase in glucose utilisation by brown adipose tissue. After cessation of treatment in the obese mice the reversal of improvements in glycosuria and brown adipose tissue activitiy followed a similar time course, supporting this hypothesis.

243. Impairment of both hepatic and peripheral glucose metabolism in the glucose intolerance of ageing

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Mechanisms of glucose intolerance with ageing were studied by simultaneously employing the forearm and double-isotope techniques to compare the responses to glucose ingestion (100 g) in 10 young (aged 20–23 years) and 10 elderly (aged 73–80 years) normal men. The elderly were markedly glucose intolerant despite normal or increased insulin responses. Basal hepatic glucose output (HGO) was reduced with age, being 12 ± 0.6 and 9.8 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in young and elderly men respectively ($p < 0.025$). Similar proportional reductions in HGO from 0–270 min after glucose loading occurred in young (56%) and elderly (52%) subjects but in the latter this fall was significantly delayed. The systemic appearance of ingested glucose (0–270 min) was slowed with age being 81 ± 3 and 64 ± 5% of the oral load in the young and elderly respectively ($p < 0.01$). In the elderly, the mean increment in total glucose disappearance from 0–270 min reached 85% of that in young men (45.6 g) but this rise was delayed, being only 53% of that in the young after 90 min. Increments in forearm glucose uptake differed less in the two groups than total glucose disappearance and were similar from 0–270 min. In conclusions, age-related glucose intolerance reflects a delayed suppression of HGO and attenuated, delayed rises in both muscle and hepatic glucose uptake despite slowed glucose absorption.

244. Beta$_2$-microglobulin: an indicator for vascular disease in diabetic and non-diabetic subjects

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Beta$_2$-microglobulin (β_2-MG) is metabolized nearly completely by tubular cells of the kidney after glomerular filtration. Since serum concentrations of β_2-MG correlates inversely with glomerular filtration rate, β_2-MG is considered to be a sensitive parameter for early diagnosis of diabetic nephropathy. In the present study, a large number of diabetic out-patients ($n = 448$) and non-diabetic control subjects ($n = 252$) was screened for β_2-MG, as well as for its correlation to serum creatinine and cardiovascular risk factors. β_2-MG was analysed by a newly developed enzyme immunoassay. The concentrations of β_2-MG in all subject without vascular disease were comparable (diabetic 1.52 ± 0.05 versus control 1.68 ± 0.05 mg/l; mean ± SEM; NS). Whereas serum creatinine in diabetic patients with and without retinopathy did not differ, a highly significant difference was found for β_2-MG: with retinopathy 1.99 ± 0.12 versus without 1.63 ± 0.05 mg/l; $p < 0.001$). Interestingly, β_2-MG was also significantly ($p < 0.001$) elevated in age-adjusted subjects (diabetic and non-diabetic) with macrovascular disease: diabetic 2.06 ± 0.08 versus 1.60 ± 0.04; control 2.17 ± 0.11 versus 1.68 ± 0.05 mg/l. β_2-MG was associated with macrovascular disease more closely than with most other well-known risk factors. It is concluded that β_2-MG might be a useful and sensitive indicator for vascular disease. However, the diagnosis of diabetic nephropathy necessitates additional diagnostic information.

245. Influence of pancreatic islet blood flow on the diabetogenic action of streptozotocin

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Exposure of the pancreatic β-cell to the β-cytotoxic agent, streptozotocin (STZ) could depend on pancreatic islet blood flow. To study this issue, rats were injected intraperitoneally with either saline, glucose, propranolol, phentolamine, yohimbine or adrenaline followed by STZ (25 mg/kg body weight, intravenously) 10 min later. Measurements of islet blood flow were performed by a microsphere technique on anaesthetized animals 10 min after injection of the drugs. Administration of phentolamine, yohimbine or glucose before STZ-injection was followed by overt diabetes, whereas propranolol- and saline-injected animals exhibited significantly lower serum glucose concentrations. The adrenaline-injected rats remained non-diabetic. Blood flow measurements showed an increase in islet blood flow 10 min after injection of propranolol or glucose while no significant effects were demonstrated by the other drugs. It appears, however, that the hyperglycaemic effect of STZ was enhanced by an increased serum insulin concentration at the time of STZ administration. It is concluded that the modulating effect of adrenoreceptor active compounds on the diabetogenic action of STZ is mediated by mechanism other than changes in the circulation, possibly at the level of the βcell itself.

246. A new mortality risk factor in Type 1 (insulin-dependent) diabetes

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The London sample for the WHO Multinational Study included 143 men and 148 women with Type 1 diabetes. By 30 June 1983, 12 men and 10 women were dead, with coronary heart disease (CHD) the acribed cause in 8 men and 5 women. Baseline measurements were correlated with mortality using a life table regression method, as the length of follow-up varied from 5 to 8 years. Significant 'predictor' variables for all causes of mortality in univariate analyses were age, systolic and diastolic blood pressures and serum creatinine for men and serum creatinine and electrocardiographic abnormality for women. For CHD death significant 'predictors' were age, systolic blood pressure and serum creatinine for men and serum creatinine for women. For all causes of death and CHD mortality, using multiple regression analysis, significant, independent 'predictor' variables were age and serum creatinine for men and serum creatinine only for women. Baseline creatinine levels in those who subsequently died ranged from 61.9 to 141.4 μmol/l. Only one death was definitely ascribed to diabetic renal disease. Thus the level of serum creatinine was a powerful in-

dicator of mortality risk, independent of age, blood pressure, serum cholesterol levels and smoking habit.

247. Oxyntomodulin, (glucagon-37), a gut peptide which potentiates glucose induced insulin release

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Oxyntomodulin (G-37) is the glucagon molecule extended by 8 amino-acids at the C-terminal part. The pancreatic content in G-37 (20% of the glucagon-like material) in rat, makes an insulin modulation possible via a paracrine pathway. The presence of G-37 in rat blood also suggests an endocrine rôle. In both cases, it was interesting to determine whether G-37 modulates insulin release. Rat pancreases were perfused at 6 or 10 mmol/l glucose. At 10 mmol/l glucose, G-37 biphasically potentiated the insulin release in a dose-dependent manner. Over baseline (499 ± 48 μU/min) G-37 induced the following insulin increments, obtained during a 5-min perfusion: 189 ± 74 μU/min, 523 ± 148 μU/min, 741 ± 96 μU/l at 3×10^{-10}, 6×10^{-10} and 9×10^{-10} mol/l, respectively. At 6 mmol/l glucose, 6×10^{-10} mol/l G-37 monophasically increased insulin release, a return to baseline being observed in the presence of G-37. Under the same conditions, a dose-response curve of glucagon on insulin release was established. In conclusion, (1) G-37, like glucagon, potentiated the glucose-induced insulin release; (2) on a molar basis, G-37 was ≃ five fold less potent than glucagon on insulín release. These data do not favour a paracrine role of G-37, but on the other hand, this gut peptide may take part in the entero-insular axis during digestion.

248. Insulin-dependent diabetes mellitus diagnosed under the age of 5 years

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Insulin dependent diabetes mellitus diagnosed under the age of five years shows an increasing incidence in the Oxford district (mean ± SD yearly age specific incidence per 10,000: 1969–1973, 0.73 ± 0.34; 1974–1978, 0.52 ± 0.19, 1979–1983, 1.26 ± 0.45). There is no similar increase in diabetes presenting in children aged between 5 and 10 years (1974–1978, 1.48 ± 0.61, 1979–1983, 1.44 ± 0.22). Over the past 17 years we have seen 64 children diagnosed under the age of 5 years. Data from this group is compared with that of children diagnosed between 5 and 10 years. All 3 out of the 64 children presenting within the first year of life were diagnosed during 1979–1983. Age of presentation is otherwise divided evenly over 1–5 years with increasing incidence not confined to any one year of age. There is no seasonal variation in time of presentation in children under the age of 5 years in contrast to the autumn/winter peak seen in those diagnosed between 5 and 10 years. In children under 5 years the duration of symptoms was < 2 weeks in 30% compared with 12% for the 5–10 year group ($p < 0.05$); 27% presented in ketoacidosis compared with 17% for the 5–10 year group (NS). Comparative data are also available between the groups for the 'honeymoon' period, insulin requirements, HLA and clinical management.

249. The effect of metabolic control on renal function in short-term streptozotocin-diabetic rats

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The effect of metabolic control on glomerular haemodynamics was investigated using a micropuncture technique in insulin-treated streptozotocin-diabetic rats. Age-matched rats served as controls. The studies were performed 2 weeks after the induction of diabetes mellitus. Kidney size; glomerular filtration rate (GFR) and single nephron GFR were substantially higher in the moderately hyperglycaemic diabetic rats (blood glucose 16 mmol/l) than in the normal animals. Single nephron GFR rose as a result of an increase in the hydraulic pressure difference across the glomerular capillary wall, caused mainly by a rise (2.4 mmHg) in glomerular pressure but also due to a reduction (0.9 mmHg) in tubular pressure. Strict metabolic control (blood glucose 5.4 mmol/l) prevented these changes. Comparing direct with indirect (stop-flow technique) measurements of glomerular pressure showed that the indirect method overestimates glomerular pressure in normal rats and in strictly controlled diabetic rats in but not in poorly controlled diabetic rats. This finding may explain the conflicting results obtained in previous studies of glomerular pressure measurements in diabetic rats.

250. Characterization of the receptors for insulin and the insulin-like growth factors on micro- and macrovascular tissue

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The role of insulin and the insulin-like growth factors (IGF) in the aetiopathogenesis of diabetic vasculopathy remains to be determined. We attempted to characterize the receptors for insulin and the IGF on micro- and macrovascular tissue. Retinal endothelial cells, pericytes, aortic endothelial and smooth muscle cells were cultured from a bovine source ^{125}I-insulin, ^{125}I-IGF-I and ^{125}I-IGF-II were chemically cross-linked using disuccinimidyl-suberate to their receptors on these cells and the receptor structure identified by SDS-PAGE and autoradiography. Using ^{125}I-insulin as tracer, a band with $M_r = 145{,}000$ and ligand sensitivity of insulin > IGF-I > IGF-II was identified on pericytes, aortic and retinal endothelial cells, but not on smooth muscle cells. When ^{125}I-IGF-I was used as tracer, a major band with $M_r = 145{,}000$ and ligand sensitivity of IGF-I > insulin > IGF-II was found on all cells. Using ^{125}I-IGF-II as tracer, a band with $M_r = 260{,}000$ was observed which exhibited a higher affinity for IGF-II than IGF-I and little affinity for insulin. These results suggest that receptors for insulin and IGF exist on both micro- and macro-vessels. The potential role these receptors may subserve in the transport of these ligands and their effect on vascular tissue metabolism remains to be determined, but could have relevance to diabetic vasculopathy.

251. Thyroid stimulating immunoglobulins in Type 1 (insulin-dependent) diabetes

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Type 1 diabetes and Graves' disease are associated and an increased frequency of HLA-D/DR3 is found in both diseases. We determined the prevalence of thyroid stimulating immunoglobulins (TSI) with both radioreceptor (TBII) and adenylate cyclase stimulation (TSAb) assays in 46 patients with Type 1 diabetes (C-peptide ≤ 0.06 nmol/l). TBII was positive in 10/46 (23%) and TSAb in 15/46 (33%) which is significantly more frequent than in non-diabetic subjects ($p < 0.01$ and < 0.001). Thyroid hormones and thyrotropin concentrations were similar in patients with and without TSI, although the mean free T_3-index (FT_3I) was 8% higher in TSI-positive patients ($0.05 < p < 0.10$). Patients with TSI required a 26% higher mean insulin dosage, despite a similar degree of diabetic control ($p < 0.03$). FT_3I was correlated to the antibody level in patients with TSAb ($R_s = 0.62$, $p < 0.05$). The demonstration of increased insulin demand in patients with TSI, a higher FT_3I in those patients and the correlation between FT_3I and TSAb point to a clinical effect of TSI in Type 1 diabetes.

252. Insulin resistance and impaired insulin responses in non-diabetic, HLA identical siblings of Type 1 (insulin-dependent) diabetic patients

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It remains controversial whether non-diabetic, HLA-identical siblings of Type 1 diabetics patients (S2H) have abnormal insulin secretion. This may partly be due to not considering the effect of insulin resistance on islet-cell function. We measured insulin sensitivity from an intravenous glucose tolerance test (300 mg/kg) using the minimal model of Bergman and related this to the maximal insulin secretory capacity estimated as the acute insulin response to arginine (5 g intravenously) determined after clamping the plasma glucose at 30 mmol/l. 12 S2H siblings showed no difference from 12 age-, sex- and weight-matched control subjects in either fasting plasma glucose (mean ± SEM 5.06 ± 0.1 versus 5.11 ± 0.12 mmol/l or basal insulin 10.8 ± 0.1 versus 11.0 ± 1.5 mU/l, respectively). Insulin sensitivity was significantly lower in the S2H siblings than in the controls (2.99 ± 0.24 versus 5.29 ± 0.58, $p < 0.01$). Absolute insulin secretory capacity was lower in the S2H siblings (210 ± 19.8 versus 255 ± 35.2 mU/l; $p < 0.05$). In non-diabetic control subjects there was a curvilinear relationship between insulin secretory capacity and sensitivity ($r = 0.63$, $p < 0.01$), and using analysis of covariance to adjust for sensitivity, the insulin secretory capacity of the siblings was much lower than that of controls ($p < 0.02$). In conclusion, compared with controls, these S2H siblings are insulin-resistant and have a low maximal insulin secretory capacity, especially when matched for insulin sensitivity.

253. Quantitive assessment of capillary numbers on fluorescein angiograms

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We describe a simple method to assess capillary loss in early diabetic retinopathy: counting the numbers of capillaries crossing lines drawn radially from the centre of the fovea on fluorescein angiograms. Fluorescein angiograms were taken in the standard manner of 10 normal subjects and 11 patients with background retinopathy. A good quality angiogram negative was projected on a 'Documentor' (Carl Zeiss Jena) at $\times 13$ magnification; an 8° field was centred on the macula; 4 fixed radius lines were drawn at 90° to each other (nasal, temporal superior and inferior) and a fifth line through the quadrant with apparently fewest capillaries. The capillaries crossing these lines were counted. Counts were omitted if vessels coincided with lines. *Results.* The mean ± SD number of capillaries crossing these lines in normal subjects was 16.3 ± 2.3, 16.9 ± 2.5, 17.5 ± 3.3, 17.4 ± 3.1; diabetic patients 12.4 ± 3.5, 11.7 ± 3.5, 11.8 ± 3.4, 12.4 ± 3.4, for the fifth line normal subjects 16.5 ± 2.0 and diabetics 10.7 ± 2.9. Total numbers for all lines in normal subjects were 81 ± 11.1 and diabetic patients 59.3 ± 13.1. Comparing respective normal with diabetic capillary counts significance was always achieved ($2P < 0.05$). This is a simple technique to quantify capillary loss in mild diabetic retinopathy: of value in assessing change with time and treatment.

254. Effects of Bordetella pertussis toxin and adenosine on insulin- and isoprenaline-stimulated glucose transport in isolated rat adipocytes

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Bordetella pertussis toxin (IAP) is supposed to block specifically the inhibitory component N_i of adenylate cyclase, whereas adenosine activates N_i. To assess further the role of adenylate cyclase and cyclic AMP in the regulation of glucose transport and insulin action, the effects of adenosine and IAP on isoprenaline- and insulin-stimulated glucose transport were studied. Glucose transport was determined with the aid of a rapid uptake assay of the non-metabolizable sugar 3-O-methylglucose. Basal as well as insulin-stimulated glucose transport were not affected when cells were pre-treated with IAP. In contrast, IAP-pre-treatment abolished the stimulatory effect of isoprenaline. Similarly, the effect of isoprenaline was antagonized by adenosine deaminase which removes adenosine spontaneously released by the cells. When IAP-pre-treated cells were exposed to a combination of insulin and isoprenaline, the stimulatory effect of the catecholamine was reversed to an inhibitory one. The results suggest that (a) the effect of insulin is unrelated to the inhibitory regulation of adenylate cyclase; (b) isoprenaline may exert both stimulatory and inhibitory effects depending on activation of N_i. The inhibitory regulation of adenylate cyclase may thus represent a pivotal link in the regulation of glucose transport.

255. Insulin gene polymorphism and susceptibility to coronary heart disease

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A large DNA insert at a polymorphic locus, 5′ to the human insulin gene, has been proposed as a genetic marker for atherosclerosis, independent of conventional risk factors. To confirm this observation, we have studied insulin gene polymorphisms in 80 Caucasoid patients undergoing coronary angiography, using restriction endonuclease Sst I and Southern blotting techniques. Fasting glucose, lipids and lipoprotein profiles were estimated at angiography and other conventional risk factors were noted. Two major insulin gene-related fragments were observed on autoradiography, corresponding to the small (class 1) or large (class 3) DNA insertion at the polymorphic locus and patients were genotyped 1/1, 1/3 or 3/3. The allelic frequency of class 3 alleles in an angiographically normal control group ($n = 20$) was 0.36; compared with 0.22 in a patient group with coronary atherosclerosis ($n = 60$). Exclusion of patients with conventional risk factors, such as hyperlipidaemia, diabetes and hypertension, did not result in a significant increase in the class 3 allelic frequency. This contrasts with earlier published findings and suggests that the class 3 allele is not a genetic marker for atherosclerosis in the population we have studied.

256. Effect of adrenaline, diabetes and insulin on rabbit skeletal muscle glycogen synthase

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Rabbit skeletal muscle glycogen synthase (GS) contains seven different sites per subunit whose phosphorylations are catalysed by three classes of protein kinases: cyclic AMP-dependent protein kinase (sites 2, 1a and 1b), calmodulin-dependent protein kinases (site 2), and independent-protein kinases (sites 2, and 3). The phosphorylation site stoichiometries were determined from GS purified from control, alloxan-diabetic and adrenaline-treated rabbits by analysis of the total phosphate content in vivo of each site after reverse phase high performance liquid chromatography separation of a complete tryptic digest of the purified GS. GS from diabetic rabbits had a twofold elevation of phosphate contents of sites 2 and 3 in vivo, which were reversed by insulin treatment. Adrenaline resulted in increased phosphorylation in vivo of site 1b (twofold), site 2 (twofold), and site 3 (1.5-fold). We conclude that the major effect of adrenaline, phosphorylation of sites 1a, 1b, and 2 are mediated by the activation of the cyclic AMP-dependent protein kinase. The mechanisms accounting for the phosphorylation of site 3 in response to adrenaline and phosphorylation of sites 2 and 3 in the diabetic state are unknown.

257. Quantitative X-ray microanalysis of pancreatic β cells irradiated by protons

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Advantage was taken of the highly sensitive proton microprobe technique for measuring various elements in the endocrine and exocrine pancreas from obese-hyperglycaemic mice. Freeze-dried pancreas sections were subjected to proton bombardment and the elemental contents in the β cells and the exocrine part were obtained from the characteristic X-rays emitted. Quantitative data were provided for 18 different elements. The molar ratio between K and Na exceeded 10, implying that neither the sample preparation nor the irradiation had incluced significant diffusion. With the demonstration of this high K/Na ratio, it seems likely that the β cells are also equipped with an efficient Na^+/K^+ pump. The β cells contained about 60 mmol Cl/l cell water. Observed amounts of Ca and Mg were equivalent to those previously recorded by electrothermal atomic absorption spectroscopy. The significant role of Zn for the storage of insulin was emphasized by the demonstration of three times as much of this element in the β cells compared with the exocrine pancreas. The sensitivity of the proton microprobe enabled measurements of various trace elements, such as Rb, Cr, Cu, Al and Pb, not previously demonstrated in the pancreatic β cell.

258. Processing of the internalized insulin-receptor complex

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^{125}I-labelled A1, B1 and B29 insulin analogues and hepatocytes were used to study the nature of the insulin-receptor complex in subcellular receptorsome and plasma membrane fractions. The latter were isolated by density gradient centrifugation after incubation of intact cells with photoprobes and subsequent ultraviolet irradiation. The fractions were analysed by SDS-PAGE to look for processing of hormone-receptor complex. Both B1 and A1 photoprobes gave labelled subunits at 135, 126, 90 and 68 K in plasma membrane. In addition, at 37 °C, but not at 18 °C, receptorsome fractions contained lower molecular weight components (< 45 K) and there was a time-dependent redistribution of label in the two highest molecular weight bands. B29 photoprobe, however, labelled only the 135 K subunit and further processing was not demonstrated. We conclude that processing of the insulin-receptor complex occurs in receptorsomes producing lower molecular weight degradation products still attached to insulin fragments containing A1 and B1 but not the B29 residue. This implies that the region including B29 is removed from the rest of the insulin molecule at an early stage following binding of insulin to receptor. A proportion of internalized insulin-receptor complex is degraded in receptorsomes and is therefore not available for recycling.

259. Teaching of diabetic patients as an important part of the treatment of diabetes mellitus – a cost benefit analysis

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We have previously demonstrated that effective teaching of diabetic patients is an important prerequisite for successful treatment. The object of this study was to investigate whether our multi-media instruction programme can improve metabolic balance in a group of 300

Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetic patients over a period of 4 years and to evaluate whether the cost of diabetic teaching is reasonable in view of the benefit obtained. Average blood glucose fell from 14.3 to 8.8 mmol/l, the average HbA_1 fell from 12.5% to 8.7% in the Type 1 diabetic patients, annual admissions to hospital dropped from 15.6 to 1.4 days. In the Type 2 diabetic group, with its wide age range and its various treatments, an improvement of metabolic state was also observed, depending on age and education. The cost of instructing one patient was estimated at oeS 1,910. The benefit per patient over the entire period of 4 years was oeS 100,000. In conclusion, these findings suggest that our multi-media instruction programme makes it possible for all age groups up to 70 years to control their long-term metabolic state and, thereby, reducing admissions to hospital and preventing long-term ill effects. It is beneficial for patients and involves little cost.

260. The value of glycosylated haemoglobin as a screening test for glucose intolerance

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To evaluate HbA_1, as a marker of glucose intolerance, we performed oral glucose tolerance tests (GTT) in 165 'normal' subjects with postprandial glycosuria. In 104 the oral GTT was normal (group A), 21 had impaired glucose tolerance (group B), 28 had diabetes (group C) and 12 had a 'lag'-type of oral GTT (group D). The mean HbA_1, value was higher in group B compared with group A but not significantly so ($6.72 \pm 0.4\%$ versus $6.23 \pm 0.1\%$ $p > 0.05$), while it was significantly higher in groups C and D than A ($7.05 \pm 0.1\%$ and $7.06 \pm 0.4\%$ versus $6.23 \pm 0.1\%$ $p < 0.001$). A weak but significant correlation was found between HbA_1, and glucose at 0, 60 and 120 min, only when all the subjects were considered together, but not in each individual group ($r = 0.265$, $p < 0.01$; $r = 0.3427$, $p < 0.001$; $r = 0.2601$, $p < 0.01$; respectively). Considering the value of 6.8% as the cut-off point, the sensitivity of HbA_1, as a screening test for diabetes detection (group C) was 76% and the specificity 74%, while as a screening test for abnormal oral GTT (groups B, C, D) the sensitivity was 59%. In conclusion, HbA_1, increases with deteriorating glucose tolerance and shows good correlation with glucose values at every point of an oral GTT, but its sensitivity as a screening test is acceptable only for the detection of diabetes and not for impaired glucose tolerance.

261. Glucose cycle in liver is increased in mild Type 2 (non-insulin-dependent) diabetes and in acromegaly

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Glucose cycle (GC) is one of the three futile cycles operating in the liver. We have measured GC in the following subjects: lean controls ($n = 7$), lean diabetic ($n = 8$), obese diabetic subjects ($n = 5$) and acromegalic subjects with normal glucose tolerance ($n = 3$). GC was estimated under basal conditions and during moderate hyperglycaemia (infusion of $2\ mg \cdot kg^{-1} \cdot min^{-1}$ of glucose during 120 min) by comparing hepatic glucose production measured with 2-^{3}H-glucose and 3-^{3}H- or 6-^{3}H-glucose. In control subjects, the activity of GC was negligible throughout the experiment ($< 0.1\ mg \cdot kg^{-1} \cdot min^{-1}$). In lean diabetic patient, GC was markedly increased during hyperglyaemia ($0.50 \pm 0.2\ mg \cdot kg^{-1} \cdot min^{-1}$; $p < 0.01$), but only slightly under basal conditions ($0.29 \pm 0.31\ mg \cdot kg^{-1} \cdot min^{-1}$). The obese diabetic patients exhibited an active GC before and during glucose infusion (0.53 ± 0.17 and $0.94 \pm 0.45\ mg \cdot kg^{-1} \cdot min^{-1}$; $p < 0.05$). In acromegalic subjects, a considerable amount of glucose was turned out through GC during normoglycaemia (1.04, 0.39, $0.41\ mg \cdot kg^{-1} \cdot min^{-1}$), as well as during the first 60 min of glucose infusion (1.13, 1.00, $0.79\ mg \cdot kg^{-1} \cdot min^{-1}$ at 40 min). In conclusion, an active GC in the liver is an early feature of Type 2 diabetes, accounting, in part, for glucose intolerance and insulin resistance. The diabetogenic effect of growth hormone is, at least partially, caused by increased activity of GC in the liver.

262. Effects of leguminous fibre in a mixed diet in Type 2 (non-insulin-dependent) diabetes

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Fifteen inadequately controlled Type 2 diabetic patients were treated during two consecutive 3-week periods in a metabolic ward. A control diet and a diet with an increased content of peas and beans (leguminous diet) were given in a random order. The diets comprised 20% of energy as protein, 33–34% as fat and the polyunsaturated: saturated ratio was 1.0. Dietary fibre contents in the control diet and the leguminous diet were 24 and 36 g/6.7 MJ respectively. The mean body weight did not change. At admission the baseline blood glucose value was 14.3 ± 0.9 mmol/l (mean $\pm$ SEM). This value decreased and was 10.2 ± 0.8 mmol/l by the last week of the control period and 9.8 ± 0.5 mmol/l by the last week of the leguminous diet period. The mean post-prandial glucose concentration at 15.00 h was significantly lower during the latter period. The mean urinary glucose concentration was lower during the leguminous diet period. The serum lipids were improved during the diet treatment irrespective of diet, but showed no significant differences at the end of the two diet treatment periods. There were no differences between the two diet periods with regard to fasting blood glucose, fasting insulin or insulin sensitivity measured by the intravenous insulin tolerance test.

263. The incidence of Type 1 (insulin-dependent diabetes) in Israeli children and adolescents in the age range 0–20 years

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A retrospective study of the entire population of Israel revealed 392 newly-diagnosed Type 1 diabetic patients, aged 0–20 years, for the period of 1975–1980. The mean annual age-specific incidence of Type 1 diabetes was 3.8×10^{-5} for the age group 0–14 years and 4.2×10^{-5} for the age group 0–20 years. The incidence among the Jews of Ashkenazi origin was 6.8×10^{-5} and that for Jews of non-Ashkenazi origin was 4.3×10^{-5}, whereas that for the Arabs was 1.2×10^{-5}. The overall incidence is lower than that reported for similar populations in most European countries, USA, Canada and New Zealand, but is similar to that reported for Arabs in Kuwait and higher only than that found in Japan. The relative importance of environmental and genetic factors in the interpopulation differences in the incidence of Type 1 diabetes remains to be established.

264. Serum C-peptide levels in patients with Type 2 (non-insulin-dependent) diabetes treated with insulin because of secondary failure to sulphonylureas

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In 150 patients with Type 2 diabetes, who developed the true secondary failure to sulphonylureas after 1–26 years of effective therapy, basal serum C-peptide was measured by radioimmunoassay using Byk-Mallinckrodt kits. At the time of investigation the patients were treated with insulin from 1 month to 19 years. In all but one patient Cpeptide was found in the serum: (1) in nine patients it was < 0.3 nmol/l (group 1); (2) in 67 patients between 0.3 and 1.05 nmol/l (group 2); (3) in 48 patients between 1.05 and 2.1 nmol/l (group 3), and (4) in 25 patients > 2.1 nmol/l (group 4). Mean daily insulin doses in these groups varied from 57 to 61 U. Linear discrimination analysis of selected variables disclosed that group 1 differed from the other groups having a lower body mass index, higher fasting glycaemia and longer insulin treatment. No significant differences were found between the remaining groups. In conclusion, in long-standing Type 2 diabetes the true secondary failure to sulphonylureas is not due always to the exhaustion of β cells. Other factors decreasing the biological effectiveness of insulin are probably also involved in the development of this phenomenon.

265. Restriction fragment polymorphisms in the major histocompatibility complex of diabetic BB rats

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Insulin-dependent diabetes in the BB-rat strain has many similarities to that in man, both clinically and immunologically. While the actual causes of the BB-rat diabetes are unknown, there is a link to the major histocompatibility complex (MHC). We examined the MHC of the diabetic BB-rat for restriction fragment polymorphisms segregating with the diabetic trait. Liver DNA was digested with various restriction enzymes, electrophoresed on agarose gels, and blotted on to nitrocellulose. These blots were hybridized with radioactive probes corresponding to either mouse class I, human class II α-chain, or human class II βchain MHC genes. Hybridization was compared in the diabetic strain and a non-diabetic control BB-rat line originating from

a common ancestral generation. We found no polymorphisms within either α- or β-chains of class II genes. Within the class I region, however, polymorphisms were seen with several restriction enzymes. A 2kb BamHI fragment appearing in DNA from each of six control rats was absent from all seven diabetic rats. Due to the complexity of the class I region, we are as yet unable to determine which gene(s) is involved. We suggest that these polymorphisms reflect a disposition towards diabetes, and that BB-rat diabetes may involve an alteration of a class I gene.

266. Impaired autoregulation of blood flow in subcutaneous tissue in long-term Type 1 (insulin-dependent) diabetic patients with microangiopathy

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Autoregulation of blood flow in subcutaneous tissue was investigated at the level of the lateral malleoulus in eight Type 1 diabetic patients with clinical microangiopathy (median age: 37 years), in eight Type 1 diabetic patients without clinical microangiopathy (median age: 34 years) and in seven healthy subjects (median age 34 years). Blood flow was measured by the local ^{133}Xenon washout technique. Reduction in arterial perfusion pressure was produced by elevating the limb above heart level. Elevation in arterial perfusion pressure was induced by head-up tilting. Venous pressure was kept constant by activation of the leg muscle vein pump (heel raisings). Arterial perfusion pressure varied between 70 and 150 mmHg. Blood flow remained within 10% of control values when the limb was elevated or lowered in the normal subjects and in the short-term diabetic patients. In all eight Type 1 diabetic patients with microangiopathy, blood flow changed almost linearly with the induced alterations in arterial perfusion pressure. Our results suggest that autoregulation of blood flow, i.e. the maintenance of blood flow within narrow limits during changes in perfusion pressure, are defective in diabetic patients with microangiopathy (arteriolar hyalinos).

267. α- and β-adrenergic regulation of ketone body kinetics and lipolysis in man

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To determine the influence of α- and β-adrenergic stimulation on ketone body kinetics (3-^{14}C-acetoacetate infusion technique) and on lipolysis, noradrenaline was infused at 80 ng $\cdot$ kg^{-1} $\cdot$ min^{-1} during 170 min either alone, or combined with phentolamine (PHEN), propranolol (PROP) or with both PHEN + PROP in groups of six to eight overnight-fasted subjects. Plasma insulin and glucagon levels were maintained constant by infusing somatostatin, insulin and glucagon. Noradrenaline increased total ketone body production by 107% ($p < 0.01$), and plasma non-esterfied fatty acid (NEFA) levels (lipolysis) by 27% ($p < 0.01$). PHEN (α-blockade) enhanced the increase in ketone production and plasma NEFA ($p < 0.01$ versus noradrenaline) whereas PROP (β-blockade) decreased both parameters to similar values as in saline infused controls. Peripheral ketone body clearance decreased by 39% during PHEN and increased by 20% during PROP, thereby enhancing the effect of altered ketone production on ketone body levels. PHEN + PROP produced similar results to PROP alone. Therefore, the increase in ketogenesis during elevated plasma noradrenaline levels, as observed during severe stress, results from the balance between β-adrenergic stimulation and α-mediated inhibition. The α-effect fails to occur in the absence of β-stimulation. The close association of lipolysis and ketogenesis suggests that adrenergic regulation of ketone body homeostasis mainly results from β-mediated stimulation and α-mediated inhibition of lipolysis.

268. Acute psychological stress does not disturb metabolic control in Type 1 (insulin-dependent) diabetic patients

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It is widely thought that acute psychological stress plays an important role in disturbing metabolic control in diabetic patients, although this question has not been investigated systematically. Therefore we have studied 10 Type 1 diabetics patients and seven age- and weight-matched non-diabetic subjects during mental arithmetic (stress 1), while delivering a speech in front of an audience (stress 2) and during leisure reading (control) on three different days. All subjects had their usual breakfast before assessment; six diabetics were studied in normoglycaemia (7.0 ± 0.7 mmol/l) and four in hyperglycaemia (23.7 ± 1.3 mmol/l). In all subjects mean heart rate and systolic blood pressure remained unchanged during control conditions, but increased by an average of 20 beats/min and 30 mmHg ($p < 0.001$) during stress 1 and by 25 beats/min and 40 mmHg during stress 2 ($p < 0.001$). Plasma adrenaline rose twofold during both stress conditions. Surprisingly, these stress responses were not associated with any deterioration of metabolic control in all diabetic patients, but glycaemia fell during all three test conditions ($p < 0.001$). Only in normal subjects did glycaemia increase by 0.6 mmol/l ($p < 0.05$) during stress 1. Ketone bodies and plasma glucagon levels remained unaffected by either stress in all subjects. In conclusion, sudden, short-lived psychological stress causing marked cardiovascular and adrenaline responses does not worsen metabolic control in Type 1 diabetes irrespective of the state of metabolic control.

269. Dietary treatment improves β-cell responsiveness to oral glucose in Type 2 (non-insulin-dependent) diabetes

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Insulin levels often seem 'normal' or 'high' in Type 2 diabetes. Ten Type 2 diabetic patients had 75 g oral glucose tolerance tests (GTT) at diagnosis. Fasting and peak plasma glucose were 14.7 ± 1.7 and 29.3 ± 2.4 mmol/l respectively (mean ± SEM). Fasting and peak plasma insulin were 15.8 ± 2.9 and 44.8 ± 7.7 mU/l. Repeat oral GTT after 6 months' treatment by diet alone gave fasting and peak plasma glucose levels of 6.4 ± 0.27 and 15.4 ± 0.9 mmol/l. Fasting and peak insulin and insulin levels at all time points during oral GTT were unchanged from diagnosis. After an overnight fast and within a week of the 6 months oral GTT, each patient had glucose infused (1–3 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}) into a peripheral vein for approximately 2 h to achieve a 'fasting' plasma glucose similar to that at diagnosis. The infusion rate was then adjusted to maintain a steady-state plasma glucose for 30 min. The 75 g oral GTT was then repeated. The 'fasting' glucose of 13.9 ± 2.0 mmol/l rose to a peak of 15.8 mmol/l ($p < 0.001$ compared to diagnosis). The 'fasting' insulin of 35.3 ± 16.1 mU/l rose to a peak of 119.3 ± 31.2 mU/l ($p < 0.05$ compared to diagnosis). Insulin levels at 15, 30, 60 and 90 min were all significantly higher than at diagnosis ($p < 0.05$). These results suggest that in untreated Type 2 diabetes, the β cell is relatively insensitive to the stimulus of ingested glucose. Dietary therapy results in an increased capacity of the β cell when the stimulus of oral glucose is given at a comparable ambient glucose level.

270. Molecular mechanisms inhibiting glucose oxidation in 48 h-starved or alloxan-diabetic rats

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Mitochondrial pyruvate dehydrogenase (PDH) complex is inactivated by phosphorylation (PDH kinase) and reactivated by dephosphorylation (PDH phosphatase). In diabetes or starvation, glucose oxidation is inhibited by increased phosphorylation of PDH complex in heart, muscles, liver, kidney and adipocytes. Increased PDH kinase activity appears to be mainly responsible and this is due partly to kinase activation by products of fat metabolism. A major mechanism of kinase activation in these conditions apparently involves the cytoplasmic synthesis of a protein factor which can be separated from the PDH complex by high-speed centrifugation (studies with inhibitors of cytoplasmic protein synthesis). Factor has been purified 300-fold and has a M_r of approximately 100,000. Activity of factor is increased 3-6-fold in heart mitochondria by starvation or diabetes. Factor activity is decreased towards normal by refeeding starved rats for 24–48 h and decreased factor activity is correlated with reactivation of near normal PDH complex activity. Insulin treatment of diabetic rats is likewise effective. Studies of factor concentration-activation relationships show that diabetes and starvation increase V_{max} and do not change $K_{0.5}$, indicative of a further mechanism over and above biosynthesis.

271. Oxyntomodulin (G-37) and glucagon (G-29): distribution in the gastrointestinal tract and the plasma of the rat

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Oxyntomodulin (glucagon-37), an intestinal glucagon-containing peptide extended at the C-terminal part by an octapeptide, is one of the glucagon-like immunoreactants (GLI). High performance liquid

chromatography associated with radioreceptor assay was used to determine the distribution of G-37 and G-29 in adult rats. The GLI concentration measured in crude extracts by radioimmunoassay (ng glucagon equivalent/g wet weight) were as follows ($n=6$): pancreas 4600 ± 900; stomach 15.2 ± 3.4; duodenum 82 ± 7; jejunum 348 ± 25; ileum 690 ± 40; caecum 248 ± 18 and colon 333 ± 12. In pancreas and stomach, the sum of G-37 plus G-29 accounted for more than 95% of the total GLI; G-37 represented 19 ± 2 and 39 ± 12 of this activity, respectively. In gut, the two peptides represented 40% of GLI and G-37 was $37 \pm 5\%$ of this activity. In peripheral plasma of fed rats, the respective concentrations of G-37 and G-29 were ($n=4$): 240 ± 60 and 83 ± 2 pg/ml. The combination of high performance liquid chromatography and radioreceptor assay allows, for the first time, the unambiguous determination of the two peptides in tissue extracts and plasma. This determination is necessary to study the physiology of oxyntomodulin, the tissue specificity of which is different from that of glucagon.

272. Inhibitors of insulin and somatomedin action in human plasma

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Following our recent demonstration that human IgG and IgM exert a potent insulin-like stimulatory effect on adipocyte metabolism, we investigated whether human plasma contains any inhibitors of insulin action. Supernatants obtained from human plasma or serum precipitated with 2.5 mol/l ammonium sulphate and dialysed repeatedly against physiological saline inhibit markedly basal and insulin stimulated adipocyte lipogenesis and glucose oxidation to CO_2. Further fractionation of the supernatant preparations by ultrafiltration revealed two consistent inhibitory fractions (10–30 KDa and 30–50 KDa). These fractions not only inhibited insulin and IgG stimulated adipocyte metabolism, but were also markedly inhibitory to basal and serum somatomedin stimulated ^{35}S uptake by porcine cartilage. These data show the presence of a low molecular weight inhibitor of insulin and somatomedin in human plasma. These inhibitors may play a role in the pathogenesis of diabetes and growth disorders.

273. Role of helper and suppressor lymphocytes and of macrophages in multiple dose streptozotocin-induced autoimmune diabetes

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Multiple streptozotocin-induced diabetes in mice serves as a model of human Type I diabetes. We have studied the possible role of T lymphocytes and macrophages in pathogenesis. The disease was induced in C57Bl/6J and C57Bl/KsJ mice (10 animals per group) by injection of 5×40 mg or 5×30 mg streptozotocin/kg body weight. C57Bl/6J mice were treated in addition with two monoclonal antibodies against I-J^b gene products (at day 8 after streptozotocin). C57Bl/KsJ mice received 2×5 mg silica particles (7 µm diameter; on days 0 and 5) or monoclonal antibody to Thy 1.2 (24 h prior to streptozotocin). Antibodies to I-J^b enhanced ($p < 0.01$) the hyperglycaemia (day 60: 23.8 ± 1.2 and 26.4 ± 1.4 mmol/l versus 17.0 ± 1.3 mmol/l after streptozotocin alone). Treatment with silica or anti-Thy 1.2 inhibited ($p < 0.01$) almost completely the development of hyperglycaemia (day 40: 11.3 ± 1.6 and 11.5 ± 1.3 mmol/l versus 19.0 ± 1.6 mmol/l after streptozotocin alone). Our data indicate that anti-I-J^b may decrease effects of suppressor T cells resulting in an augmentation of hyperglycaemia. Elimination of T cells with anti-Thy 1.2 and inactivation of macrophages with silica particles leads to an inhibition of hyperglycaemia. It is suggested that helper T lymphocytes and macrophages are responsible for β-islet cell destruction. The disease process apparently is controlled by suppressor T lymphocytes.

274. Ciglitazone Reverses cyclic AMP-induced post-insulin-receptor-resistance in isolated rat adipocytes

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Ciglitazone (5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione) has a hypoglycaemic effect only in insulin-resistant hyperglycaemic (non-insulin-dependent diabetes) animal models, but not in normal animals. As cyclic AMP causes insulin resistance at the receptor and post-receptor levels in isolated rat adipocytes, we investigated whether ciglitazone could reverse these effects. Mono-A14-(^{125}J) insulin binding and insulin stimulated 3-0-methyl-glucose transport were measured in isolated rat epidydimal fat cells kept in Krebs-Ringer-Hepes buffer (pH 7.4, 37 °C, 16 mmol/l glucose, 2.5 g/dl bovine serum albumin). Ciglitazone (< 5 µmol/l) had no effect on basal or insulin-stimulated glucose transport, but reversed the inhibitory effect of pre-incubation with isoprenaline (10 µmol/l, 30 min). There was no reversal of the isoprenaline (10 µmol/l) induced inhibition of insulin binding and no effect on insulin binding itself. Therefore, ciglitazone seems to counteract cyclic AMP effects at a post-insulin binding level. Further experiments indicate that ciglitazone stimulates the translocation of glucose transporters into the plasma membrane.

275. Glucose oxidase enzyme electrode for rapid determination of plasma glucose

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The performance of a commercially available glucose-measuring system (Radelkis, Hungary) was improved using an enzyme probe of our own construction. Further, the measuring procedure and data processing were optimized. The assay consisted of subsequent additions of 100 µl portions of glucose standard and two identical unknown samples. Readings of the glucose measuring system, indicating decrease of current after addition of glucose, were loaded into a pre-programmed calculator to determine mean ± SD of four measurements (two per sample). Electrode response was linear in the glucose concentration range of 1.5–15.0 mmol/l and reproducible within 3% for our enzyme probe and 4% for the Radelkis probe. Glycaemias obtained with both electrodes were verified spectrophotometrically and a close correlation was found (our probe: $r = 0.998$; $p < 0.001$ and the Radelkis probe: $r = 0.988$; $p < 0.001$). Response velocity of our probe to a step change in glucose concentration was substantially higher than that of the Hungarian probe ($\tau_{90\%} = 22.5 \pm 4.0$ versus 45.5 ± 3.7 s). After buffer exchange the response stabilized within 45 s with our probe and 300 s with the Radelkis probe. Thus, only our probe was usable for such frequent glycaemia monitoring as required during an euglycaemic clamp. Quadruplicate readings for glycaemia were available and the system was ready for a new cycle at 4.5 min intervals. One enzyme membrane is stable for 1 year or 1500 assays.

276. Morphometric analysis of the endocrine pancreas in BB/Hagedorn rats before the onset of diabetes

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Insulin-dependent diabetes develops spontaneously between 60–140 days of age in 80–90% of BB/Hagedorn (BB/H) rats. The β-cell volume before clinical diabetes was determined in a prospective study at the ages of 15 days (10 BB/H, 6 BB control), 30 days (6 and 6) and 45 days (6 and 6) in diabetes-susceptible BB/H and non-diabetic BB rats (from the w-subline). Pancreatic endocrine cells stained by immunocytochemistry were quantitated by computerized morphometry. No difference was found in the parenchymal volume of the pancreas at age 15 days (0.10 versus 0.10 ml, mean). Whereas lower volumes were found in BB/H rats at days 30 and 45 (0.29 and 0.50 versus 0.33 and 0.62, respectively). The absolute β-cell volume was reduced in the BB/H compared to BB controls rats; (ml $\times 10^{-3}$) day 15, 0.65 versus 0.87; day 30, 1.39 versus 1.47; day 45, 2.37 versus 3.49, respectively. Although the groups did not differ statistically, the β-cell volume was lower than the lowest individual β-cell volume in the BB control group in 3/6 BB/H rats, both on days 30 and 45. The results suggest that β-cell volume is decreased in many diabetes-susceptible BB rats at least 30 days before clinical onset of diabetes.

277. Immunogenetic differences between substrains of BB rats

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Since the discovery of insulin-dependent diabetes in BB rats substrains of these animals have been bred in several laboratories. At present an important question is whether and to what extent differences exist between these substrains. To clarify this question, we studied the serological reaction at first of normal sera to 'natural' antibodies reacting with rat major histocompatibility antigens (RT1) and secondly of hyperimmune sera produced in RT1 congenic rats against RT1 antigens of individual BB rats of various substrains (BB/OK, BB/PhiK, BB/DK). To determine the RT1 antibodies we used the dextran haemagglutination assay against red blood cell donors of five differ-

ent RT1 haplotypes. 'Natural' RT1 antibodies were detected in 19.5% of BB/DK, 17.8% of BB/PhiK and 0% of BB/OK rats. The existence of 'natural' RT1 antibodies was not related to the metabolic state of rats. The reaction patterns of hyperimmune sera produced against RT1 antigens of individual BB/OK rats were found as in congenic rat strains, whereas the sera produced against individual BB/DK rats were characterized by an unexpected immunological reaction, so far only found in antisera gained from recombinant rat strains (LEW.WR1, LEW.WR2). The differences observed between BB rat substrains suggest a strongly genetic heterogeneity among the BB rats.

278. Decay of the response to covalently bound insulin in adipocytes

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Lipogenesis is stimulated in adipocytes when their insulin receptors are covalently cross-linked to photosensitive insulin derivatives by ultraviolet irradiation. This stimulation decays with time in a manner consistent with endocytosis. Decay half-times were determined for active covalent complexes with 2-nitro, 4-azido-phenylacetyl (Napa) derivatives of insulin B2 (Napa)des-Phe^{B1}-insulin (B2) and B29 (Napa)insulin (B29), under various conditions. Lysosomotropic agents (methylamine, ethylamine, chloroquine) increased the half-time (with 200 µmol/l chloroquine, $\tau = 156$ min; normal $\tau = 78$ min, 37 °C, pH 7,4) but led to increased basal rates of lipogenesis in control cells. Temperature reduction also slowed the decay. Although the effectiveness of B2 and B29 complexes differ, and increase with pH, no differences between their decay rates were observed at any pH. Similarly the decay was independent of the degree of receptor loading within the limits studied. Half-times decreased linearly with increasing pH ($\tau = 95$ min, pH 6,9; $\tau = 55$ min, pH 8,1). The mechanism responsible for the decay – conceivably internalization – is thus pH-sensitive, but not dependent on the level of covalent occupancy. The two systems appear to be different. Our results further suggest that normal ranges of insulin concentrations are unimportant in determining the turnover rate of insulin receptors.

279. Success in reducing the rate of ketoacidosis in patients treated with continuous subcutaneous insulin infusion

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In a feasibility study of continuous subcutaneous insulin infusion (CSII) in an outpatient clinic, ketoacidosis was seen at a much higher rate in a pump-treated group (group A) compared with groups using intensified conventional therapy (group B) or unintensified regimes (group C) during the first year of the study. Rates were: – group A: 0.14 episodes/patient year; group B: 0.008; group C: 0.01. Hence ketoacidosis occurred at a 17.5 times greater rate with CSII than with intensified conventional treatment. Most episodes occurred at times of physical stress, mainly infections. Identification of this problem enabled the risk to be stressed to the patients and explicit guidelines to be given of action to be taken at such times, including glucose and ketone monitoring and extra insulin dosage. Consequently, in the second year, the ketoacidosis rate in group A was more than halved to 0.06 episodes/patient year with rates of 0.03 in group B and 0.03 in group C. The reduction in group A may in some part be ascribable to some less careful patients discontinuing CSII. In conclusion, identification of increased risk of ketoacidosis in a CSII-treated group led to additional educational steps resulting in a halving in the rate of the problem, though it remains double that occurring with intensified conventional treatment.

280. Involvement of the autonomic nervous system in the in vivo thyrotropin-releasing hormone-induced increases in plasma levels of glucagon, insulin and glucose in rabbits

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Thyrotropin-releasing hormone (110 nmol TRH) increased plasma levels of glucagon (97 ± 9 µmol/l), insulin (55 ± 9 mU/l) and glucose (3.8 ± 0.6 mmol/l) in rabbits ($n = 4$). However TRH has no direct effect on pancreatic hormone release in man in vivo or in rats in vitro. The aim of the present study was to investigate whether the effects of TRH in rabbits were mediated by the autonomic nervous system. The '110 nmol TRH Roche-induced hyperglucagonaemia' was inhibited by phentolamine (an α-receptor blocking drug, 42 ± 16 µmol/l, $p < 0.05$), yohimbine (an α-2-receptor blocking drug, 37 ± 6 µmol/l, $p < 0.05$) and atropine (46 ± 10 µmol/l, $p < 0.05$). The '110 nmol TRH-induced hyperinsulinaemia' was inhibited by propranolol (a β-receptor blocking drug, 6 ± 1 mU/l, $p < 0.05$). The '110 nmol TRH-induced hyperglycaemia' was inhibited by all four drugs (1.0 ± 0.2 mmol/l, 0.3 ± 0.03 mmol/l, 1.1 ± 0.1 mmol/l, 0.7 ± 0.1 mmol/l, respectively, $p < 0.05$). The effects of TRH on plasma levels of glucagon, insulin and glucose cannot be explained by increases in the plasma levels of catecholamines, since adrenaline, noradrenaline and isoproterenol show different effects on plasma levels of glucagon, insulin and glucose from TRH. It is concluded that TRH, given intravenously to rabbits, probably acts on regions in the central nervous system which control carbohydrate metabolism and the release of glucagon and insulin from the endocrine pancreas by activation of the sympathetic and parasympathetic nervous system.

281. Activation by insulin of phospholipase C in fat cells: relationship to activation of pyruvate dehydrogenase

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Incubation of fat cells with exogenous phospholipase C (PHL-C) leads to activation of pyruvate dehydrogenase (PDH), similar to that observed with insulin. Therefore, we have examined whether insulin might elicit its effect on PDH by activation of endogenous PHL-C. PHL-C activity was measured in homogenates prepared from rat adipocytes after incubation in the presence or absence of insulin using [^{14}C]-labelled or unlabelled L-α-phosphatidylinositol as substrate. There was a clear increase of PHL-C activity of insulin-treated cells (40.9 ± 6 mU/mg protein, $n = 26$, $p < 0.0005$) compared with control cells (18.9 ± 2.1 mU/mg protein, $n = 26$). Addition of insulin to the homogenates remained ineffectual. Activation of PHL-C and PDH showed striking similarities with respect to (1) the insulin dose-response relationship with an apparent K_a-value of 5–10 mU/l, (2) the time course reaching maximal activation after 10 min, (3) lack of an insulin effect in the absence of medium glucose and Ca^{2+}. Our results suggest that in fat cells insulin exerts control on phosphatidylinositol metabolism by activation of PHL-C. This may be related to the mechanism of PDH activation and perhaps other metabolic effects of insulin.

282. Mechanism of the dawn phenomenon in Type 1 (insulin-dependent) diabetes: enhanced hepatic glucose production rather than impaired utilization

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The mechanism of the 'dawn phenomenon' was studied in 12 C-peptide negative diabetic patients (aged 30 ± 2 years) treated with continuous subcutaneous insulin infusion. From 04.00 to 08.00 h, 10/12 patients demonstrated a > 0.5 mmol/l rise in blood glucose from 4.6 ± 0.4 to 6.2 ± 0.7 mmol/l ($p < 0.01$), when a constant basal infusion (11.8 ± 1.2 mU·kg^{-1}·h^{-1}) was used. In these patients the rate of glucose production (Ra, 2.14 ± 0.04 mg·kg^{-1}·min^{-1}, 3-^{3}H-glucose infusion) exceeded the rate of utilization (Rd, 1.89 ± 0.03 mg·kg^{-1}·min^{-1}, $p < 0.02$). When the patients were restudied and the infusion rate was stepwise increased by 38 ± 9% between 24.00 and 08.00 h, Ra fell to 1.75 ± 0.03 mg·kg^{-1}·min^{-1} ($p < 0.01$), equal to Rd (1.84 ± 0.03 mg·kg^{-1}·min^{-1}) and the phenomenon was abolished (5.0 ± 0.7 versus 4.9 ± 0.7 mmol/l). In eight healthy control subjects, Ra (1.66 ± 0.02 mg·kg^{-1}·min^{-1}) matched Rd (1.71 ± 0.03 mg·kg^{-1}·min^{-1}) and blood glucose remained unchanged. Peripheral free insulin levels in diabetics were similar during constant (12.0 ± 0.05 mU/l) and variable (11.0 ± 0.04 mU/l) infusions and higher than in controls (5.0 ± 0.05 mU/l, $p < 0.005$). Plasma cortisol, glucagon and growth hormone levels were comparable in controls and diabetics during both studies. In conclusion, (1) the dawn phenomenon is due to excessive rate of glucose production rather than impaired utilization; (2) a step-up in the overnight insulin delivery prevents the dawn phenomenon by reducing hepatic glucose production; (3) this may be associated with enhanced clearance of insulin.

283. 'Single cell infiltration' of pancreatic islets: an alternative concept of β-islet cell destruction

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The classical definition of insulitis or isletitis in recently diagnosed Type 1 (insulin-dependent) diabetes describes the occurrence of areas of mononuclear infiltration, recognizable by light microscopy. The same holds true for isletitis in partially analogous animal models (the BB-rat, the non-obese diabetic mouse, the NZB-mouse or low-dose

streptozotocin diabetes in mice). Interestingly, infiltration areas are mostly peri-insular and periductular rather than intrainsular. We report on ultrastructural studies which demonstrate the existence of a second type of isletitis, the 'single cell infiltration'. We have studied isletitis induced by low-dose streptozotocin administration in C57BL6/J mice and spontaneous isletitis and periductilitis in autoimmune NZB-mice. Before the occurrence of classical isletitis with large areas of infiltrating lymphocytes, we observe a phase with single macrophage and lymphocyte infiltration associated with β-islet cell destruction. The 'single cell infiltration' is recognizable only by electron microscopy and persists during later classical multicellular and mostly peripheral islet infiltration. Classical isletitis may be a secondary reaction as observed in delayed-type hypersensitivity reactions.

284. ACTH potentiates growth hormone-induced insulin insensitivity in Type I (insulin-dependent) diabetes

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We have demonstrated previously that a short-term infusion of growth hormone (GH) induces a long-standing decrease of insulin sensitivity in diabetes. Now we have investigated the influence of ACTH in this respect. We studied 14 male, non-obese diabetic patients after an overnight fast. Subcutaneous insulin administration was discontinued 24 h before study and replaced by an intravenous infusion of insulin. Insulin sensitivity was measured by blood glucose monitoring during a combined infusion of somatostatin (100 µg/h), insulin ($0.0004\ U \cdot kg^{-1} \cdot min^{-1}$) and glucose ($3\ mg \cdot kg^{-1} \cdot min^{-1}$) between 09.00 and 15.00 h. GH (0.02 IE/kg intravenously for 0–60 min) and ACTH (0.1 mg intravenously bolus at start) were given separately or together. Plasma levels of glucagon were similar in all study periods and endogenous GH was continuously suppressed by somatostatin. Free insulin reached the same plateau in all experiments. Plasma levels of cortisol followed the diurnal pattern in experiments without ACTH. Exogenous ACTH elevated plasma cortisol by approximately 400 nmol/l after 3 h, returning to basal at 6 h. Exogenous GH raised blood glucose significantly after 4 h and this effect was potentiated by ACTH, while ACTH alone did not modify insulin sensitivity. We conclude that episodic increase of circulating GH, resembling the response to hypoglycaemia, induces a long-standing decrease of insulin sensitivity and that this effect is markedly potentiated by ACTH.

285. Equipotency of continuous and pulsatile hormone administration on hepatic glucose production in vitro

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To elucidate the efficacy of continuous versus intermittent exposure to adequate stimuli, such as glucagon and insulin, hepatic glucose production (HGP) was monitored in response to hormone infusion. To this end isolated livers of fed rats were perfused with 5 mmol/l glucose Krebs-Ringer buffer in a non-recirculating system. Using this model it was shown that intermittent exposure (3 min on/off intervals; dose 50%) to glucagon (3.5×10^{-11} mol/l) elicited the same rise in HGP (1.60 ± 0.52 mmol/100 min; mean ± SD) as continuous exposure to the same hormone concentration (1.65 ± 0.24 mmol/100 min). When insulin (100 mU/l) was infused to suppress glycogenolytic glucagon action (7×10^{-11} mol/l), the same phenomenon was seen as continuous and intermittent (3 min on/off intervals) insulin administration inhibited glucagon stimulated HGP (3.14 ± 0.14 mmol/100 min) to the same extent (Δ-55% versus Δ-57%). Doubling the 'off' period to 6 min and thereby reducing the dose to 33% did not diminish inhibition by insulin of glucagon-stimulated HGP (Δ-49%). These results demonstrate that pulsatile glucagon as well as insulin administration is associated with an equipotent hormone action even if the hormone load is reduced by 50–66%. From this we conclude that pulsatile hormone administration is preferable to and most likely more physiological than continuous infusion.

286. The concomitants of death from diabetic nephropathy: 10-year follow-up study in Warsaw

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In a cohort of 4553 diabetic patients, aged 18–68 years, with diabetes lasting 1–10 years at onset, 49 (3.5%) died from diabetic nephropathy of a total of 1405 deaths traced during 10-year period of study; the condition rated fifth among main causes of mortality. For every nephropathic-deceased subject two others died during the period of study and two surviving patients were chosen, matched for sex and 5-year age group. The following baseline variables were compared between the groups under investigation: duration of diabetes, relative body mass, systolic and diastolic blood pressure, fasting glycaemia, plasma creatinine, total serum cholesterol and lipids, proteinuria and glycosuria. The nephropathic group differed significantly from the two other groups in fasting glycaemia, total serum lipids and plasma creatinine ($p < 0.01$ for each). The duration of diabetes was longer among nephropathic deaths, followed by other deaths, and it was shortest among survivors; the same was true for glycosuria. No significant difference was found in the base-line measurements of systolic and diastolic blood pressure, relative body mass, cholesterol and proteinuria. In conclusion, the risk of death from diabetic nephropathy was most closely related to base-line hyperglycaemia, and ensuing renal insufficiency.

287. Effect of exogenous insulin, glucagon, adrenaline, oestrogen and transplantation on hyperphagia in chronic alloxan- and streptozotocin diabetic rats

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The effect of exogenous insulin (regular, protamine Zn, Interdep) in doses of 1–40 U/kg, subcutaneously (SC), glucagon (0.05 µmol/kg, SC), adrenaline (0.2 µmol/kg SC) and oestradiol (5 mg/kg SC), as well as the effect of successful cure of diabetes by transplantation of neonatal pancreases, were studied in chronic alloxan- and streptozotocin-diabetic male and female rats with hyperphagia of up to twofold the food intake in normal rats. Insulin, administered for five consecutive days in doses up to 40 U/kg, did not altered diabetic hyperphagia, while cessation of insulin administration, causing an increase in glycaemia and glycosuria, depressed hyperphagia. In comparison with normal rats, glucagon and adrenaline caused a shorter (1–2 h) depression of food intake as did oestrogen after a longer latency (3–4 days). Rats cured from diabetes by renal-subcapsular homo (allo)-transplantation of neonatal pancreases, still exhibited hyperphagia for about 2 weeks after restoration of normoglycaemia and return of body weight to pre-diabetic levels. In conclusion, the glucostatic mechanisms of food intake regulation are functional in diabetes, but less suppressible by glucagon, adrenaline and oestrogens. This is presumably because of predominance of hyperphagic mechanisms stimulated by catabolism and loss of body mass.

288. Variations of very low molecular weight growth peptides in sera from Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetic patients and healthy subjects

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Serum growth peptides (Mol. wt. < 2000) for human vascular cells occur in Type 2 diabetic patients and are more potent than insulin or growth hormone. As the specificity of these growth peptides for Type 2 diabetes is unknown, sera from five Type 1 and 25 Type 2 diabetic patients and from 25 healthy subjects were compared. Human fibroblasts were cultured in DME + 10% of the respective sera for 5–7 days. Their growth effect (cell number/plate) was compared with the dialyzed serum fraction (Mol.wt. > 3500), the respective serum dialysate (Mol.wt. < 3500) and with the effect of pretreatment with pronase (200 µg/ml, 4 h, 25 °C). Removal of the dialyzable serum fraction reduced the growth effect of sera from Type 1 and Type 2 diabetic patients by 44% and 37% ($p < 0.005$) but that of normal sera by 8% only ($p < 0.01$). In contrast, there was no difference between the three dialyzed serum types. Recombination with the serum dialysate restored the growth effect of untreated serum. Tenfold concentration of serum dialysate form sera from Type 2 diabetic patients stimulated cell growth up to 243% and that from normal serum up to 150% ($p < 0.01$). Protease pre-incubation of Type 1 diabetic serum dialysate abolished its growth effect. In conclusion, sera from all diabetic patients contained increased amounts of growth peptides of very low molecular weight for vascular cells that could contribute to the increased risk of angiopathy in diabetes.

289. Heterogeneity of in vivo insulin sensitivity among individual tissues: studies using the euglycaemic clamp in the rat

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The variation of in vivo insulin sensitivity among individual target tis-

sues and their relationship to whole body insulin sensitivity has not been established. We have examined this using the euglycaemic clamp in the conscious rat. By administering boli of ^{3}H-2-deoxyglucose and ^{14}C-glucose during a 2 h clamp with subsequent sacrifice and tissue analysis, we obtained insulin response curves for glucose metabolic rate (Rg′estimated from ^{3}H-2-deoxyglucose phosphorylation) and/or glucose storage products (fractional ^{14}C in glycogen or lipids). 29 studies were performed at five insulin levels. Half-maximal insulin sensitivities, obtained by computer fitting, for Rg′ in primarily oxidative muscle were: soleus (80 mU/l), red gastrocnemius (150 mU/l) and diaphragm (150 mU/l). These were similar to a whole body (i.e. glucose infused to maintain euglycaemia) sensitivity of 133 mU/l. Other muscle types were less sensitive (white gastrocnemius 280 mU/l and extensor digitorum longus 320 mU/l). Adipose tissue was more sensitive (sensitivity for both Rg′ and ^{14}C into lipids of 60 mU/l). Insulin did not increase liver lipid or glycogen storage. Thus there is marked heterogeneity of in vivo insulin sensitivity among different target tissues and even among muscle types. Whole body insulin sensitivity is similar to that found in oxidative muscle.

290. Effect of porcine gastric inhibitory polypeptide on β-cell function in Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes

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The slow and low insulin response to a meal in diabetic patients may in part be due to a lack of effect on the βcells of gastric inhibitory polypeptide (GIP). The effect of highly purified natural porcine GIP on C-peptide release was examined in six Type 1 diabetic patients (mean age 31 ± 3 years), six Type 2 diabetic patients (mean age 61 ± 2 years) and six normal subjects (mean age 32 ± 3 years). All were normal weight. At −120 min a glucose or insulin infusion was started aiming at a constant plasma glucose of 8 mmol/l. On two separate days GIP (2 pmol · kg^{-1} · min^{-1}) or NaCl were infused from zero to 30 min. Mean plasma glucose from −30 to 180 min were not different on the two days (GIP: 7.90 ± 0.05 mmol/l, NaCl: 7.89 ± 0.09 mmol/l) or between the groups. The first 10 min of GIP infusion increased plasma GIP to 141 ± 10 pmol/l (a physiological post-prandial level). Concomitantly C-peptide increased significantly in all three groups. From 10 to 30 min, GIP rose to reach supraphysiological levels (259 ± 15 pmol/l). During the same period there was a further significant C-peptide increase in the normal but not in the diabetic subjects. Physiological concentrations of porcine GIP caused an immediate β-cell response in all three groups. Higher levels of GIP further increased β-cell function in the normal but failed to do so in the diabetic subject.

291. Detection of islet cell antibodies by indirect immunofluorescence using pancreatic sections of different species: diversity of antigens and antibodies

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Using pancreatic tissue of different species for the determination of islet cell antibodies (ICA) allows the demonstration of structural variety of the target antigens and of binding peculiarities of the individual patient sera. By means of indirect immunofluorescence, patient sera from Type 1 (insulin-dependent) diabetics 13 males, 10 females, all positive on human pancreas sections of an HLA-typed donor of blood group 0) were examined using specimens of pancreas from the monkey (Cynomolgus, Makake), horse, wild duck. IgG – and IgM antibodies could be demonstrated on both cryopreserved and Bonin-fixed tissues. Two distinct patient groups were found: (A) sera positive on all targets, ICA persisting for a longer time; (B) ICA positive only in the initial phase of disease, with negative results on all targets at a later stage. The persisting antibodies were specific for multiple targets. Concordance of results was seen with the highest frequency between horse and human pancreas (23/32, 72%). Sections of fresh frozen horse pancreas are suitable for the detection of IgM-ICA. There is evidence for different pathogenetic forms of Type 1 diabetes. The presence of the target antigen in all examined tissues permits the hypothesis that in the development of Type 1 diabetes the primary event is autoaggression against a physiological determinant of β cells.

292. Role of cyclic AMP and Ca^{++}-dependent proteins in the control of insulin release

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Cyclic AMP and Ca^{++}-dependent proteins (calmodulin and synexin) are believed to control insulin release. We studied their relative roles in first and second phase insulin release, using inhibitors of adenylate cyclase (RMI 12330), and of calmodulin (trifluoperazine, TFP) and synexin (promethazine, PMZ). In 60 min incubations of rat islets, all agents inhibited glucose-induced (16.7 mmol/l) insulin release dose-dependently with ID_{50} 10 μmol/l for RMI, 14 μmol/l for TFP and 5 μmol/l for PMZ. At 5 min incubation, 14 μmol/l TFP abolished both insulin and cyclic AMP responses to glucose. The phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX, 0.1 mmol/l) partially restored both effects of glucose. At 5 min, PMZ (40 min preincubation) had no effect on glucose-induced insulin and cyclic AMP responses, despite marked inhibition of insulin release at 60 min. RMI at 5 min had modest inhibitory effects on insulin response and cyclic AMP generation (latter NS) which was further reduced by IBMX. Results with PMZ show that synexin does not control cyclic AMP generation; it may inhibit non-cyclic AMP mechanisms which influence second phase release. Effect of TFP indicates that calmodulin may be acting mainly through cyclic AMP generation. These results suggest a major role for cyclic AMP in the control of early insulin response to glucose.

293. Reduced urinary excretion of TXB_2 and PGE_2 in Type 1 (insulin-dependent) diabetes

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Prostanoids are known to be important modulators of haemodynamic function in the kidney. To evaluate their potential role in the diabetic renal hypertrophy-hyperfunction syndrome, PGE_2 and TXB_2 were measured (radioimmunoassay after silicic acid chromatography) in the 24 h urines of seven Type diabetic women (duration of the disease: 13 ± 3 years) compared with six control women, and in six Type 1 diabetic men (duration: 7 ± 2 years) compared with nine control men. None of the patients displayed proteinuria, abnormal renal function or hypertension. The urinary excretion (μg/24 h – mean ± SEM) of TXB_2 was decreased in the diabetic women (161.6 ± 21.0 versus 246.8 ± 22.9, $p < 0.02$), as in the diabetic men (156.7 ± 38.8 versus controls 286.7 ± 17.1; $p < 0.01$). The urinary PGE_2 were also decreased in the diabetic women (130.1 ± 26.8 versus 249.3 ± 39.4; $p < 0.05$). Urinary PGE_2 is not a reliable index of the renal biosynthesis in men because of seminal contamination. Among various parameters, including glycaemia, microlbuminuria and creatinine clearance, prostaglandin excretion was only correlated with HbA_{1c}. In conclusion, renal PGE_2 and TXB_2 are decreased in Type 1 diabetes, which should be added to the already known abnormalities of prostanoids in diabetes. Prostaglandins, especially TXA_2, played an important role in the renal haemodynamic changes in various kinds of glomerulonephritis and could be one of the mediators of the early haemodynamic changes featuring diabetes.

294. Complications of long-term intraperitoneal insulin infusion

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Encouraged by excellent metabolic control achieved with the low-risk intraperitoneal (IP) access for insulin infusion through externally-worn devices used continuously for more than 2 years, we chose the IP route with implantable devices in 46 patients. All have now had a minimum of 6 and a maximum of 24 months of such treatment; all have stabilized and shown improved metabolic control. 37 still show good metabolic control; two are stabilized but need increasing doses of insulin, seven deteriorated after 6–18 months. Of the seven, five have had laparoscopy showing tissue growth, and three of the five have had a histological examination. All three show local amyloid in the inner layer of the tissue wall which encapsulated the catheter. Insulin flow was stopped in one patient; two showed normal flow at routine pump check but an altered distribution pattern in the sequential images, performed with Tc-99m-pertechnate. Tissue growth leads to encapsulation of IP catheters. Formation of local amyloid and/or insulin degradation can occur even when insulin flow remains unimpeded. These facts may limit use of the IP route.

295. Effect of continuous subcutaneous insulin infusion and intensified conventional therapy on peripheral and autonomic nerves

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Nine Type I (insulin-dependent) diabetic patients (age: 18–32 years) received continuous subcutaneous insulin infusion (CSII) for 4 weeks and subsequently intensifed conventional therapy (ICT) to compare the effects of both treatment regimens on peripheral and autonomic nerves in a self-controlled study. Motor and sensory nerve conduction velocity (NCV), H-reflex, E/I-, R30/R15-, and Valsalva-ratio were measured before and 1, 2, 4, 6, 10 weeks, and 6 months after the beginning. During CSII motor NCV improved 6× (>3 m/s), sensory NCV 5× (>5 m/s), H-reflex-latency 1×, E/I 7×, Valsalva-ratio 8× and R30/R15 5×. The time course of improvement varied considerably. During ICT, NCV and H-reflex-latency did not change, E/I and Valsalva-ratio decreased significantly (4× and 5× respectively). Six months later sensory NCV and R30/R15 had declined 3×, E/I 2×, and Valsalva-ratio 4×. HbA_1 before treatment and the decrease in HbA_1-concentration during the study are inversely correlated to the increase in motor NCV ($p<0.01$, $p<0.05$). E/I improved significantly earlier than Valsalva-ratio ($p<0.03$). In conclusion, CSII improves nerve function. The extent of increase in motor NCV depends on the degree of metabolic control before CSII therapy. Parasympathetic nerves seem to demand less time for recovery than do sympathetic ones.

296. Preparation of a natural human proinsulin standard

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Human pancreatic proinsulin from two different side-fractions obtained during the preparation of insulin from human pancreases was purified by cation exchange, gel filtration, anion exchange and reversed-phase liquid chromatography (HPLC). The final purified products had an amino-acid composition which agreed well with the known composition of proinsulin, although tyrosine was 29% lower than expected. The molar concentration of proinsulin in stock solutions was calculated on the basis of the concentration of at least eight of the amino-acids in the proinsulin. Sequence analysis of amino-acids 1–39 showed a single peptide chain having the expected sequence. Reversed-phase HPLC showed no difference between the proinsulin in the two preparations and biosynthetic human proinsulin from Lilly. Vials containing 10 pmol proinsulin in 1 ml phosphate-albumin buffer were made for all three materials. The two natural proinsulins and the biosynthetic human proinsulin showed identical standard curves in radioimmunoassays for proinsulin, C-peptide and insulin. However, the standard used hitherto for human proinsulin radioimmunoassay was found to be approximately three times weaker. Consequently we recommend that one of the three new standards be used.

297. An animal model of peripheral and portal insulin delivery from transplanted islets

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For an animal model of Type 1 (insulin-dependent) diabetes to be valid in assessment of the metabolic responses to islet transplantation, absolute deficiency of endogenous pancreatic β-cell function must be documented, and the integrity of the transplanted tissue demonstrated. As streptozotocin (65 mg/kg) fails to produce complete and irreversible β-cell destruction in the rat, we have used 150 mg/kg. Animals are then absolutely insulin-dependent, but no side effects or tumours were seen in 50 rats kept 4 months from injection. Immunohistochemistry of the pancreas demonstrates a reduction in islet number, absence of β cells, but preservation of glucagon- and somatostatin-secreting cells. After 4 months, histology of syngeneic cultured fetal islets transplanted into the splenic pulp or under the renal capsule demonstrates healthy tissue embedded in a vascular-fibrous stoma and associated with proliferating adipose tissue. Islet microarchitecture is preserved, with central β cells surrounded by peripheral A cells. Animals given 3000 islets had normal fed and fasting blood glucose levels and weight gain, but insulin-sensitive adipose tissue lipoprotein lipase activity was reduced (13.7 ± 2.1 versus 28.5 ± 1.7 (normal rats) $\mu mol \cdot h^{-1} \cdot g^{-1}$; $p<0.001$) in the fed state, and blood glucose rose abnormally under stress. However animals transplanted with 5000 islets were normal in these respects.

298. Pre-conceptional insulin pump treatment of pregnant diabetic women

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Since the beginning of 1982, insulin-dependent diabetic women who wanted to become pregnant have been allocated to either insulin pump treatment (CSII) or unchanged conventional therapy (UCT). CSII-treatment has been initiated at least 2 months prior to conception and maintained throughout the entire pregnancy. Until now, nine pregnancies have been completed in the CSII-group (White classes B-F) and 10 pregnancies in the UCT-group (White classes B-D). Six weeks after conception the mean ± SEM HbA_{1c} was 7.0 ± 0.2% in the CSII-group and 7.2 ± 0.5% in the UCT-group (NS). These low HbA_{1c} levels were maintained throughout pregnancy in both groups. After 261 ± 2 days, 10 healthy infants (one pair of twins), with a mean birth weight of 3480 ± 115 g, were born to the CSII-treated women and after 258 ± 4 days, 10 healthy infants, with a mean birth weight of 3765 ± 1127 g, were born to the UCT-treated women. No malformations or severe neonatal morbidity were observed in either group. In conclusion, pre- and post-conceptional CSII-treatment of insulin-dependent diabetic women is safe and efficient, but (at least in a specialized centre for diabetic pregnancy) it does not so far seem to be superior to UCT.

299. Effect of ischaemia on peripheral nerve function in diabetes mellitus: results with a simple non-invasive method

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Effect of ischaemia on the mixed nerve function of the median nerve was investigated in 29 diabetic patients, 8 normal subjects and 3 islet cell antibody-positive subjects before the onset of diabetes. A pneumatic cuff was applied around the upper arm and the pressure was raised above systolic pressure to produce ischaemia, and median nerve action potentials were recorded by surface electrodes at the elbow. In normal subjects, amplitudes of nerve action potentials (ANAP) decreased rapidly during ischaemia and values after 15 min of ischaemia were 34 ± 19% (mean ± SD) of the initial levels. However, in diabetic patients ANAP decreased gradually and after 15 min ischaemia was 96 ± 28% of the initial levels. All 29 diabetic patients, including three insulin-dependent diabetic patients with <1 year of diabetic history had values greater than the normal range. Values for HbA_{1c} were not correlated with those for ANAP. The three islet cell antibody-positive subjects before the onset of diabetes had normal ANAP. These results suggest that an abnormal resistance to ischaemia is the earliest manifestation of peripheral nerve dysfunction in diabetes and our method is simple, non-invasive and useful in the investigation of nerve dysfunction.

300. Inter-laboratory insulin antibody workshop: a report of a collaborative study in Europe and the United States*

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Comparing results of insulin antibody measurements made in different laboratories has been difficult because of different methods and reporting units. Sixty-eight serum samples (17 from non-diabetic and 51 from insulin-treated subjects) were divided into aliquots and distributed blind to 12 laboratories. Assay separation methods included second antibody (3), cellulose adsorption (1), immunoelectrophoresis (2), charcoal (1) and polyethylene glycol (5). Mono-iodinated A-14 insulins from one source were provided to all the laboratories. Within-laboratory correlation for results using pork and human tracer was high (>0.98); for beef and human tracer low (0.73–0.93). Between-laboratory rank correlation was good; Spearman rank coefficients ranged from 0.84 to 0.99 (median 0.94). General conclusions were (1) the use of standardized methodology and label was an advantage; (2) polyethylene-glycol and second-antibody methods were equivalent; (3) some assays were more prone to the effects of prior insulin removal than others, and (4) 'percentage bound' would be the most convenient reporting unit. (* Participants: C. Binder, K. Federlin, E. Fineberg, L. Kerp, V. Kruse, D. Kumar, A. Kurtz, J. Lutterman, J. Palmer, J. Poortman, G. Reeves and G. Schernthaner)

301. Increased chylomicronaemia in fat fed rats given gastric inhibitory polypeptide antibodies

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It has been suggested that gastric inhibitory polypeptide (GIP) has a

role in the clearance of chylomicron triglyceride. The effect of GIP antibodies on the removal of circulating lipid following an oral triolein load was investigated in rats. The immunoglobin fraction of a sheep anti-GIP serum (α-GIP) and a normal sheep serum (NSS) were prepared. Twelve, fasted, male Wistar rats (mean weight 175 g) were dosed orally with 1 ml triolein and injected intraperitoneally with 50 mg of either α-GIP or NSS and bled from the tail at hourly intervals for 5 h. GIP antibodies were detected in excess in all test group samples after time zero. Mean immunoreactive insulin concentrations remained unstimulated throughout. Basal triglyceride values were 1.30 ± 0.21 (α-GIP) and 1.22 ± 0.14 (NSS) mmol/l (mean $\pm$ SEM). At 2 and 3 h these rose to 1.85 ± 0.33 and 2.23 ± 0.13 (α-GIP) versus 1.24 ± 0.09 and 1.46 ± 0.07 (NSS) ($p < 0.1$ and 0.0005 respectively) at these time points. The mean area under the curve for the two groups showed a significant difference ($p < 0.05$), as did analysis of variance ($p < 0.01$). The results support the suggestion that endogenous GIP may have a role in the absorption and/or clearance of dietary triglyceride.

302. Association of low serum HDL and HDL_2-cholesterol with coronary heart disease in non-insulin-dependent diabetes

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A low serum HDL and HDL_2 cholesterol concentration is one of the characteristic serum lipid abnormalities in non-insulin-dependent diabetes. In this study, serum lipid and lipoprotein levels were measured in 284 non-insulin-dependent diabetic patients (139 males, 145 females) aged 45–64 years. Altogether 43 (27 males, 16 females) had a previous myocardial infarction (MI) verified at hospital, or ECG abnormalities diagnostic of previous MI. Both male and female patients with MI had significantly lower levels of HDL and HDL_2 cholesterol ($p < 0.001$ for males, $p < 0.01$ for females) than diabetic subjects without MI. Female diabetics with MI had also higher levels of total, LDL and VLDL triglycerides than female diabetic patients without MI, but such a difference was not observed among male diabetics. The difference in HDL and HDL_2 cholesterol concentration between diabetics with and without MI did not depend on age, smoking, physical activity, alcohol intake, obesity, duration of diabetes or glycosylated haemoglobin A_1. If, in addition, adjustment was made by analysis of covariance for the total triglyceride concentration, the difference in HDL and HDL_2 cholesterol level between the groups of diabetic patients with and without MI disappeared in females, but not in males. In conclusion, even among non-insulin-dependent diabetic patients with generally low HDL and HDL_2 cholesterol concentrations the presence of MI is associated with a particularly low HDL and HDL_2 cholesterol level.

303. Time-course of cytotoxic islet cell antibodies in the BB rat

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Sera from 20 BB rats from three diabetes-prone litters, and from 12 non-diabetes-prone Sprague-Dawley rats were collected twice a week from 60–160 days of age and tested for complement-dependent cytotoxicity to ^{51}Cr-labelled rat islet cells in vitro. All sera from rats which subsequently became diabetic ($n = 14$) and glucose-intolerant ($n = 2$) were toxic to islet cells in the presence of complement. Sera from non-diabetic BB rats ($n = 4$) tended towards greater cytotoxicity than controls, although this did not reach significance. The maximal cytotoxic potency occurred either before or immediately after the onset of diabetes. This potency varied considerably in sequential samples from each rat. The sera which had proved toxic to islets did not lyse hepatocytes in vitro. In the presence or, sometimes, in the absence of complement, these sera suppressed the B cell responses of normal islets to stimuli, but not the A and D cell responses to arginine. The time-course of immunoglobulin binding to islet cells, as detected by an ELISA technique, was similar to that of cytotoxicity to ^{51}Cr-labelled islet cells. These results suggest the presence of one or more antibody capable of binding to B cells, inhibiting their function and inducing lysis.

304. Alteration of colorimetric strip reading in diabetic subjects

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Impaired colour vision is a common feature in diabetes. The aim of this study was to evaluate whether this could interfere with colorimetric strip reading by diabetic patients. 75 diabetic and 25 non-diabetic subjects were asked to read strips of six urine tests and one blood test commonly used in daily practice, by matching a set of strips to the coloured reference chart of the tests used. Each subject made 36 strip readings, and strips were identical for every subject. Diabetic patients made significantly more errors than non-diabetics (mean rank of cumulated errors 460 versus 421 respectively, $p < 0.01$), and diabetic patients with retinopathy made more errors than diabetics without (mean rank of cumulated errors 368 versus 319 respectively, $p < 0.001$). There was no difference between diabetic subjects with background or with proliferative retinopathy. A greater proportion of errors was observed with certain test strips in both groups. This was correlated with measurements of the spectral reflection coefficient of each colour; for those tests incriminated, differences of wavelength fell under the physiological discriminative threshold of the retina. In conclusion, diabetic patients are less reliable than non-diabetics on strip reading, and those with retinopathy are more prone to errors. Certain tests basically do not allow for accurate reading.

305. Impaired glucagon release during hypoglycaemia in Type I (insulin-dependent) diabetes – importance of blood glucose level

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The glucagon response to hypoglycaemia, but not to other stimuli, is blunted in Type I diabetes and a negative correlation has been found between the glucagon release and duration of diabetes. The reason for this insensitivity to a change in the ambient glucose concentration is presently unknown. This study evaluates the effect of a short-term elevation of the blood glucose levels on glucagon release during subsequent hypoglycaemia. The glucagon response during insulin-induced hypoglycaemia was studied in six healthy volunteers following 2 h of euglycaemic (5.0 mmol/l, E) or hyperglycaemic (18.0 mmol/l, H) glucose clamp. The same glucose nadir was reached in both studies (E: 1.5 ± 0.1, H: 1.5 ± 0.2 mmol/l). Also the insulin levels were similar at glucose nadir and during the compensatory period. The glucose recovery rate was delayed and the maximal glucagon release was significantly less after hyperglycaemic clamp (E: 101 ± 25, H: 54 ± 7 pg/ml, $p < 0.05$). However, the maximal release of adrenaline, cortisol and growth hormone were similar. Thus, the release of glucagon during hypoglycaemia is influenced by the previous blood glucose level. This finding may be one reason for impaired glucagon release in Type I diabetes.

306. Induction therapy of non-insulin-dependent diabetes: insulin sensitivity

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The addition of phenobarbitone (PB) to sulphonylurea treatment may improve glucose control in non-insulin-dependent diabetes. A change in insulin sensitivity may be involved. To evaluate the problem, we investigated nine healthy volunteers using the euglycaemic clamp technique before and after a 10 day course of PB therapy. The glucose disposal rate increased from 5.95 ± 1.63 to 7.91 ± 1.47 $mg \cdot kg^{-1} \cdot min^{-1}$ ($p < 0.001$) and the immunoreactive insulin level decreased from 19.1 ± 1.47 to 15.2 ± 4.2 mU/l ($p < 0.02$), whereas fasting blood glucose levels and body weight remained unchanged. The data demonstrate that PB increases insulin-mediated glucose metabolism. The findings suggest a new approach to influencing insulin sensitivity in man.

307. Measurement of insulin-dependent pH changes in peripheral mononuclear blood leucocytes by flow cytometry

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Receptors for insulin on mononuclear leucocytes (MNL) have been demonstrated by binding studies with labelled hormone. However, it is unclear to what extent the presence of receptors is correlated with the functional response of MNL to insulin. The aim of this study was to investigate insulin-induced pH changes in freshly isolated MNL (Ficoll, medium L 15, 37 °C) with a Fluvo-Metricell flow-cytometer using the pH-sensitive dye 1, 4-diacetoxy-2,3-dicyano-benzene. Effects of insulin (30 U/l) and/or glucose (5 mmol/l) were measured in non-diabetic, Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetic females ($n = 3$ each) on the single cell level by simultaneous determination of intracellular pH, cell volume, esterase activity and cell viability in kinetic studies. Insulin combined with glucose caused a rapid increase of pH in monocytes of all subjects ($\overline{\Delta}pH_{Co} = 0.17 \pm 0.03$, $\overline{\Delta}pH_1 = 0.22 \pm 0.02$, $\overline{\Delta}pH_2 = 0.42 \pm 0.08$) within

3 min as well as in lymphocytes of Type 2 diabetic patients ($\Delta pH_2 = 0.15 \pm 0.03$). In monocytes, alkalisation was also induced by incubation with insulin alone ($\Delta pH_{Co} = 0.29 \pm 0.04$, $\Delta pH_1 = 0.11 \pm 0.02$, $\Delta pH_2 = 0.23 \pm 0.04$). Glucose alone seemed to decrease pH in monocytes ($\Delta pH_{Co} = 0.89 \pm 0.14$, $\Delta pH_2 = 0.84 \pm 0.05$, t = 20 min) and in lymphocytes ($\Delta pH_{Co} = 0.46 \pm 0.05$, $\Delta pH_2 = 0.32 \pm 0.04$), without showing any effects on MNL of Type 1 diabetic patients. Determination of rapid changes of pH in MNL of probands with different metabolic disorders could reflect the functional responsiveness of cells to insulin in the diabetic state. Furthermore, it could provide more information on the transcellular ion-exchange which is possibly correlated with a 'second messenger' signal.

308. Thrombophilic diathesis already present before diabetic microangiopathy

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The aim of this study was to measure parameters of fluid phase coagulation and platelet function and to compare these factors in nonage-matched and age-matched healthy control subjects ($n = 25/n = 14$) and diabetic patients with ($n = 21/n = 19$) and without ($n = 28/n = 12$) microangiopathy. Under basal conditions, serum glucose, glycosylated albumin, HbA_1, C-peptide and glucagon values were significantly different between controls and diabetics, but not between diabetics with and without late complications. Fibrinogen, factor VIII antigen and antithrombin III were significantly higher in diabetics, even more in those with late complications. F-CB 3 was markedly elevated in diabetic patients with late complications only. Fibrinopeptide A values showed large variations and were not significantly different in the groups tested. Platelet aggregation induced by ADP, collagen and arachidonic acid was higher in diabetics especially in those with late complications. Thromboxane B_2 production, under aggregatory conditions, was not different between healthy and diabetic subjects. In conclusion, platelet hyperaggregability and some parameters of hypercoagulability are already present in diabetic patients without late complications and may therefore play a rôle in the development of microangiopathy.

309. Glycosylated-erythrocyte-membrane-proteins and metabolic parameters in insulin-dependent-diabetic subjects

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Glycosylation process was reported also to involve erythrocyte-membrane-proteins (GEMP) in diabetes. However no data exist about a correlation between GEMP and the metabolic parameters in diabetic patients. To verify whether there is a correlation between GEMP and metabolic parameters, 12 Type 1 (insulin-dependent) diabetic patients (aged 37 ± 2.9 years; mean $\pm$ SEM), and 10 controls subjects (aged 36 ± 4 years) were studied. GEMP, glycosylated serum proteins (GSP), glycosylated haemoglobin (HbA_{1c}), fasting plasma glucose (FPG) were evaluated in all subjects. Plasma glucose profile, glycosuria, ketonuria were also evaluated in the diabetic patients. Mean $\pm$ SEM GEMP were 11.3 ± 0.7 nmol hydroxy-methylfurfural (HMF)/mg protein versus 4.90 ± 0.3 in control subjects; GSP were 0.98 ± 0.05 nmol HMF/mg protein versus 0.50 ± 0.05 in control subjects ($p < 0.001$); HbA_{1c} was $6.6 \pm 0.4\%$ versus $4.7 \pm 0.2\%$ in control subjects ($p < 0.005$); FPG was 9.95 ± 1.50 versus 4.72 ± 0.17 mmol/l in control subjects ($p < 0.005$). GEMP were positively correlated with GSP ($r = 0.77$; $p < 0.01$), HbA_{1c} ($r = 0.73$; $p < 0.01$), FPG ($r = 0.72$; $p < 0.01$), mean daily plasma glucose ($r = 0.73$; $p < 0.01$) and highest daily plasma glucose ($r = 0.79$; $p < 0.001$) in diabetic patients. In conclusion, glycosylated erythrocyte membrane proteins are significantly increased and related to other metabolic parameters in Type 1 diabetes.

310. Insulin and glucagon secretion from perfused A-cell-rich splenic bulbs of duck pancreas

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Glucagon and insulin secretion were evaluated in the A-cell-rich perfused splenic bulb of duck pancreas. Stable basal levels were observed with glucose (11 mmol/l), corresponding to normoglycaemia, and both secretions were stimulated by arginine and 3-isobutyl-1-methylxanthine, demonstrating the validity of their technique. In spite of a slight increase in insulin release, glucose (33 or 55 mmol/l) did not significantly affect glucagon secretion. However, A-cell inhibition occurred when high, but physiological amounts of insulin (50 mU/l) were infused together with glucose (33 mmol/l). So the perfused splenic bulb of duck pancreas can be identified as a natural insulin-deficient model and should prove to be a useful system in the study of A-cell regulation. Arginine directly acts at the pancreatic level and cyclic AMP probably plays a key rôle in the regulation of glucagon secretion. The results are compatible with the view that insulin is involved in the inhibition of A-cell secretory activity in response to an increase in glucose concentration, in an endocrine rather than a paracrine way.

311. Morphological findings in placentae of insulin-dependent diabetic patients treated with continuous subcutaneous insulin infusion

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Twenty-two placentae from patients (White Class B-R) treated with continuous subcutaneous insulin infusion (CSII) were studied. In 10 patients CSII was started before conception. In three cases available placental material was studied from pregnancies previous to CSII treatment. Light microscopy of the placentae showed villous oedema, hypo- or hypervascularity, syncytiotrophoblast necrosis, prominent cytotrophoblast, prominent Hofbauer cells and increased fibrin. Electron microscopy showed presence of 'blebs', focal syncytial necrosis, increased syncytial secretory/absorptive activity, thickening of basal membrane, increased collagen, endothelial proliferation (villous capillaries) and immature stromal cells. These morphological findings can be grouped under three headings: placenta dysmaturity (as indicator of placental maturation pathology), fetal vessel pathology and placental ischaemia. The morphological changes do not differ from those already reported in overt diabetes mellitus and controlled gestational treatment. No significant differences between pre- and post-conceptional CSII were seen. Comparison with placentae from previous pregnancies showed no notable differences. The presence of these findings strongly suggests that tight metabolic control achieved with CSII does not affect the actual morphological expression of the disease in pregnant Type I (insulin-dependent) diabetic patients.

312. Phosphatidylethanolamine N-methylation and insulin release in isolated pancreatic islets of the rat

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Rat pancreatic islets methylate phosphatidylethanolamine (PE) lipids to form phosphatidylcholine (PC) with S-adenosyl-L-methionine (AdoMet) as the methyl donor. Islet homogenate PE-N-methyltransferase activity had pH optima at 6–7 and 8–9, was not cation-dependent, and was inhibited by S-adenosyl-L-homocysteine, sodium deoxycholate and Triton X-100. Addition of PE, phosphatidyl-N-monomethylethanolamine (PMME), and phosphatidyl-N, N-dimethylethanolamine (PDME) enhanced [^{3}H]methyl incorporation into PMME, PDME, and PC, respectively. Enhanced PDME and PC methylation in the presence of monomethylethanolamine or dimethylethanolamine suggested the coupling of base exchange enzyme activity. Isoproterenol (Iso) (10^{-4} mol/l), but not glucose (17 mmol/l), stimulated phospholipid methylation in islet homogenates. Propranolol inhibited the Iso effect. Intact islets utilized the AdoMet precursor [^{3}H]methionine, and glucose or Iso stimulated phospholipid methylation and insulin release. Iso potentiated, to a similar extent, glucose-stimulated methylation and hormone release. Islet phospholipid methylation was enhanced by 8-bromo-cyclic GMP (0.2 mmol/l) by up to 64%, but was not affected by 2-deoxy-glucose, tolbutamide nor 8-bromo-cyclic AMP. 3-Deazaadenosine inhibited both glucose and Iso-stimulated methyl-transferase activity and insulin release. Propranolol inhibited the β-adrenergic potentiation of glucose-induced phospholipid methylation and insulin release. These data suggest that PE-N-methyltransferase plays a role in amplification of the islet cell stimulus-secretion coupling response to certain secretagogues.

313. Selective exercise of soleus muscles from obese and lean mice: effects on glucose metabolism and insulin responses

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Physical exercise in vivo may enhance insulin sensitivity and glucose utilization. To evaluate the effect of muscle exercise per se, we investigated basal and insulin-stimulated glucose metabolism in exercised soleus from lean mice and mice with insulin-resistant obesity induced

by gold thioglucose. Selective exercise of soleus muscle was induced in one leg by tenotomy of the gastrocnemius 4 days before muscle isolation; the controlateral soleus was used as a control. Basal deoxyglucose uptake and glycolysis increased markedly in exercised muscles from both lean and obese animals. These alterations were accompanied by a tenfold increase in fructose 2,6-bisphosphate content. In the presence of maximally effective insulin concentrations, deoxyglucose uptake and glycolysis were identical in exercised and control muscles from lean mice. In muscles from obese animals, however, exercise and insulin exerted partly additive effects. Insulin sensitivity and insulin binding were not altered in exercised muscles from either lean or obese animals. In conclusion, selective exercise, restricted to one muscle, enhances basal glucose utilization. In muscles of obese mice (but not of lean animals) exercise per se increases the response to maximal insulin concentrations without modifying the altered insulin sensitivity and decreased insulin binding.

314. Stimulatory and inhibitory effects of L-leucine upon glucagon secretion by the perfused rat pancreas

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L-leucine has variously been reported to stimulate, not to affect or to inhibit glucagon secretion. Therefore, the effect of L-leucine, in three different concentrations (0.2, 2.0 and 15.0 mmol/l), upon glucagon secretion by the perfused rat pancreas was examined at various glucose levels and in the absence or presence of other amino-acids (L-arginine and L-glutamine). In the presence of glucose alone (3.3 mmol/l), L-leucine caused a dose-related increase in glucagon output. This positive response was markedly reduced at a higher glucose concentration (8.3 mmol/l). In the presence of arginine (5.0 mmol/l) and at a low glucose concentration (3.3 mmol/l), L-leucine provoked a transient increase in glucagon output followed by a dose-related, sustained and reversible inhibition of glucagon release. Likewise, L-leucine (15.0 mmol/l) reversibly inhibited glucagon secretion evoked by L-glutamine (10.0 mmol/l) in the absence of glucose. Thus, L-leucine can either stimulate or inhibit glucagon secretion. The stimulatory effect of L-leucine is most evident in the presence of glucose alone, in low concentration, whereas its inhibitory effect prevails when glucagon secretion is already stimulated by amino-acids, such as L-arginine or L-glutamine. L-leucine may affect glucagon output even when used at a physiological concentration.

315. The effect of an aldose reductase inhibitor (sorbinil) on diabetic neuropathy

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We studied the effect of 100 mg sorbinil/day on peripheral nerve conduction, symptoms, signs and erythrocyte sorbitol concentration in diabetic polyneuropathy. The study was a double-blind placebo controlled cross-over trial divided into four 4-week periods. Sorbinil was given either in the second (group 1) or fourth period (group 2). Initially 43 patients were recruited and 31 completed the trial. Comparisons were made between the measurements at the end of placebo run-in period and both treatment periods. Compliance was assessed by measuring plasma sorbinil concentration and counting returned capsules. There was no statistically significant differences in symptoms, signs or nerve conduction variables. Erythrocyte sorbitol concentration decreased significantly during the sorbinil period (mean ± SEM group 1: 21.65 ± 2.04 versus 12.61 ± 1.93 nmol/g Hb; group 2: 19.81 ± 2.30 versus 9.94 ± 2.32 nmol/g Hb; $p < 0.001$). There was no correlation between the fall in erythrocyte sorbitol concentration and other parameters. One patient developed rash, myalgia and fever, which subsided in 2 weeks. There were no other significant adverse effects. These results indicate that sorbinil can decrease intracellular sorbitol concentration. However, convincing evidence of the efficacy of sorbinil was not found.

316. Diabetic macroangiopathy associated with Dupuytren's disease

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In a survey on the prevalence of Dupuytren's disease, carpal tunnel syndrome, tenovaginitis stenosans and stiff hand syndrome, the hands of 144 consecutive diabetic patients and 96 non-diabetic control subjects were examined by standard methods. 74% of diabetic versus 28% of control subjects ($p < 0.001$) showed abnormalities. Although most abnormalities were confined to low-grade Dupuytren's disease, the average severity, as also indicated by percentage of previous operative corrections, was significantly increased in the diabetic group. Prevalence increased with age and duration of diabetes. Sex and type of diabetes had no influence. The association between stiff hand syndrome and retinopathy, which has been reported by Rosenbloom in young diabetic subjects, was probably present in our adult population, but did not reach statistical significance ($0.05 < p < 0.1$). Of more importance seems to be the strong correlation ($p < 0.001$) that exists between Dupuytren's disease and macroangiopathy (myocardial infarction, coronary bypass surgery, severe peripheral arterial disease). It is postulated that this relation is due to a common metabolic alteration (i. e. glycosylation) of connective-tissue molecular elements both in the hand and in the vascular wall. Thus, Dupuytren's disease of the hand may be a mirror of diabetic macroangiopathy.

317. Diabetes mellitus and changes in fatty acid composition of adipose tissue

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The fatty acid composition of subcutaneous adipose tissue of the anterior abdominal wall was examined by gas-liquid chromatography in 20 diabetic patients, 8 with Type 1 (insulin-dependent) and 12 with Type 2 (non-insulin-dependent) diabetes. Compared with the control group (20 healthy subjects on the same dietary regimen regarding the dietary intake of fats), the examined group was found to have a significantly higher percentage of total saturated fatty acids (13%, $p < 0.0025$), mostly palmitic acid (17%, $p < 0.0005$), and a significantly lower percentage of total polyunsaturated fatty acids (24%, $p < 0.005$). This reduction resulted from the significantly low content of linoleic acid (29%, $p < 0.005$). Contrary to these observations, among polyunsaturated acids, a significant increase was found in eicosatrienoic acid (53%, $p < 0.05$), which could be correlated with the rapid development of the atherosclerotic process in these patients. The altered ratio between palmitic and linoleic fatty acid represents an important characteristic of the above changes. The following values for the control group were obtained by the method of analysis of variance of 16 : 0/18 : 2 ratio: mean 1.04; in Type 1 diabetic patients: mean 1.78, $LSD_{1\%} = 0.38$; in Type 2 diabetic patients: mean 1.25, $LSD_{10\%} = 0.22$.

318. Markers for the development of insulin-dependent diabetes may not 'co-exist'

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Identical twins of insulin-dependent diabetic patients have a 50% risk of developing diabetes within 5 years. Possible markers of future diabetes include islet cell antibodies (ICA), increased levels of activated T-lymphocytes (Ia positive) and decreased first-phase insulin response to an intravenous glucose challenge. We studied 12 non-diabetic co-twins of diabetic patients diagnosed within the last 5 years. Of these, only one was positive for both ICA and Ia, showed a decreased first-phase insulin release and had impaired glucose tolerance. Of the remainder, two had impaired glucose tolerance, 3 of 10 had ICA (cytoplasmic and complement fixing), 4 of 6 were Ia-positive and 2 of 7 had decreased first-phase insulin release. However, these markers often did not co-exist. Thus, some twins had impaired oral glucose tolerance with a normal insulin response to intravenous glucose; or were Ia positive yet ICA negative; or showed ICA with normal insulin response and finally could have deficient insulin response yet be both Ia and ICA negative. This demonstrates the uncertainty of these features as markers of impending diabetes.

319. Longitudinal study of cytotoxic antibodies and inhibitory lymphocytes in seven diabetic children during the first year of their disease

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To investigate the possible role of autoimmune phenomena in the pathogenesis of the remission period in Type I (insulin-dependent) diabetes, we have studied the time-course of two immunological markers in seven diabetic children (aged: 1.5–7 years). During 12 months following diagnosis, we determined: (1) cytotoxic islet cells antibodies (ICA) by the release of ^{51}Cr from mice islet cells after complement fixation and (2) inhibitory lymphocytes tested by the inhibition of mice islet cell insulin secretion after patient lymphocyte addition. All the children were treated by a restricted diet and two

injections daily of highly purified pork insulin. Five out of seven children underwent a remission period (daily insulin requirements < 0.5 U/kg) within the first weeks of treatment, which period persisted in three after one year. ICA were positive in 85% of the children at the time of diagnosis, but decreased after 6 months of disease (10% of positive sera at 12 months). ICA titration was not stable in any one patient. Inhibitory lymphocytes were found in 85% of the children at onset and remained elevated (80% at 12 months), despite a slight decrease after 6 months of disease. No correlation was found between the daily insulin requirements and immunological parameters. In conclusion, circulating ICA were present in diabetic children with high frequency immediately after diagnosis. This frequency decreased after 6 months. Inhibitory lymphocytes were also present immediately after diagnosis. They appeared to be more stable during the first year of disease.

320. Assessment of the reproducibility of cardiovascular autonomic tests in diabetic patients

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The aim of our study was to evaluate the reproducibility of five tests exploring both orthosympathetic and parasympathetic functions in diabetes. Ten insulin-dependent diabetic patients (seven males and three females; mean age 32.9 years, range 19–49 years) were examined five times on five different days by the same investigator and under the same basal conditions. The following tests were performed every day: heart rate variation on deep breathing; Valsalva manoeuvre; lying to standing; blood pressure response to standing; sustained handgrip test. The coefficients of variation (CV) showed that the most reproducible tests were lying to standing (CV 3%, range 0.9–8%) and Valsalva manoeuvre (CV 7%, range 2–19%). Although the deep breathing test was highly sensitive, it resulted in a mean CV of 13% (range 2–43%). Substained handgrip and orthostatic hypotension were more variable and less reproducible than the other tests, having respectively low CV of 21% and 76%, respectively.

321. Prevention of diabetes in the BioBreeding/Worcester (BB/W) rat with cyclosporin

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Diabetes mellitus occurs spontaneously in 40–60% of BB/W rats between 60–120 days of age. An autoimmune pathogenesis is suggested by the prevention of diabetes with both immune suppression and enhancement therapies. The fungal metabolite cyclosporin acts by blocking activation of effector cells or sparing suppressor T-lymphocytes. Laupacis, et al. prevented BB diabetes with life-long cyclosporin treatment. We wished to determine whether short duration treatment with cyclosporin prevents diabetes, thyroiditis and autoantibody synthesis. Animals received cyclosporin: orally (1 mg/100 g body weight), dissolved in olive oil; intraperitoneally (2 mg/100 g body weight), dissolved in Intralipid. Control rats received olive oil or Intralipid. Ten consecutive doses were given between 30–40, 40–50, 50–60, 60–70 and 70–80 days of age. Oral cyclosporin reduced diabetes only among animals treated between 60–70 days (7/30 versus 22/30, $p < 0.001$). The larger dose of intraperitoneal cyclosporin reduced diabetes among all groups treated before 70 days of age (18/113 versus 64/118, $p < 0.001$). Diabetes was significantly delayed in all cyclosporin-treated rats ($p < 0.01$). Neither thyroiditis nor autoantibody synthesis were reduced among cyclosporin-treated groups. In conclusion, activation of effector lymphocytes responsible for β-cell destruction may be blocked by short duration cyclosporin administration. The long-lived protection against diabetes contrasts with the absence of protection against thyroiditis and autoantibody synthesis.

322. L-aromatic amino-acids as potentiators of insulin release: role of decarboxylase

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Substrates for L-aromatic amino-acid decarboxylase: – L-5-hydroxytryptophan (L-5-HTP), *M*-tyrosine, and *O*-tyrosine, strongly potentiate glucose-induced insulin release. The present study on *ob/ob*-mouse islets shows that these compounds also potentiate insulin release induced by other secretagogues. L-5-HTP (4 mmol/l) increased the effect of 1 μmol/l glibenclamide or 20 mmol/l D,L-glyceraldehyde, and the potentiating effect of 4 mmol/l L-5-HTP, 10 mmol/l D,L-*M*-tyrosine, or 10 mmol/l D,L-*O*-tyrosine on insulin release induced by 20 mmol/l L-leucine was inhibited by the decarboxylase blocker benserazide (0.1 mmol/l). L-tryptophan, above 4 mmol/l, potentiated the effect of 10 mmol/l D-glucose, whereas L-phenylalanine and L-tyrosine did not. The inhibitor of amino-acid metabolism, amino-oxyacetate (1 mmol/l), reduced the effect of 10 mmol/l L-tryptophan, but 0.1 mmol/l benserazide did not. L-5-HTP-potentiation of glucose-induced insulin release was inhibited by benserazide but was not affected by amino-oxyacetate. Benserazide had no effect on the release induced by 20 mmol/l L-leucine + 10 mmol/l L-glutamine or 10 mmol/l ketoisocaproate + 10 mmol/l L-glutamine. The stimulatory effect of decarboxylase substrates thus differs from that of several other amino-acids in being sensitive to benserazide. These findings support the hypothesis that insulin release can be stimulated through increased decarboxylase activity.

323. Urinary excretion of an apparently novel polysaccharide in male Type I (insulin-dependent) diabetic patients

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Urinary excretion of non-dialysable conjugates of glucose was increased significantly in 50% of male and 17% of female diabetic patients. Among 38 male diabetics the increase was greater in those with Type 1 (74%) than in those with Type 2 (non-insulin-dependent) diabetes (20%). There was no correlation with patient age, known duration of diabetes, or urinary glucose or protein excretion. A high-M_r (91,000–104,000) glucoconjugate was purified from urine of 10 male patients (fractional precipitation, ion exchange chromatography, gel filtration, electrophoresis). The material contained conjugated glucose, galactose and sometimes mannose but not peptides, amino-acids or amino sugars. In three diabetics showing excretion rate > 20 times normal, 60% of conjugated glucose was recovered; galactose and mannose were $< 2.5\%$ glucose. The material was not a known glucan (resistant to glucosidases; not bound by Concanavilin A sepharose). In four patients with excretion rates 2–4 times normal and two normal men, recovery of conjugated glucose was 11–34% and the material contained an excess of conjugated galactose. It is suggested that male Type I diabetic patients exhibit increased excretion of a novel glucan of post-glomerular origin. When excretion is high (> 20 times normal) M_r is high; when it is low, lower M_r forms may predominate and be lost during fractional precipitation.

324. Studies of cryopreserved adult human islets of Langerhans

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Studies of islet isolation from human pancreas have shown a low yield of viable islets. Multiple donors may be required for islet transplantation, and a means of accumulating sufficient histocompatible tissue will be required. We have therefore developed a method of cryopreservation of adult human pancreas. Tissue was obtained at operation and from cadaver renal donors. The pancreas was distended with buffered-bicarbonate and chopped into fragments. These were incubated in increasing concentrations of dimethyl sulphoxide (DMSO), cooled at a rate of 0.3 °C/min to −40 °C, then placed directly into liquid nitrogen, and stored for up to 4 weeks. Tissue was thawed rapidly in a 37 °C bath and the DMSO diluted out in a sucrose solution. Viability of frozen-thawed tissue was assessed by light microscopy, using both conventional and immuno-peroxidase staining, electron microscopy, and insulin secretory response. Intact islets with normal morphology, normal complement of insulin storage granules and other organelles were demonstrated. An enhanced insulin secretory response to 20 mmol/l glucose + 5 mmol/l theophylline was observed. Cryopreservation of adult human islets of Langerhans therefore appears to be feasible. Problems still remain with the isolation of large numbers of islets from either fresh or frozen pancreas.

325. Polarization of islet cells: evidence from asymmetric budding of enveloped viruses in monolayer cultures of endocrine pancreas

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Pancreatic islet cells are not randomly distributed within islets of Langerhans, but are arranged in a precise topographical pattern. However, manifest polarization, as found in pancreatic exocrine cells and other secretory epithelia, is lacking in islet cells. To explore the possibility of a functional polarization of islet cells, we infected monolayer cultures of endocrine pancreas with enveloped RNA vi-

ruses known to bud selectively from either the basolateral (VSV) or the apical (influenza virus) plasma membrane domains in polarized epithelial cells. Extensive budding was observed in electron microscopy 3.5–4.5 h, and 8.5 h after infection for VSV and influenza virus, respectively. A quantitative evaluation on thin sections perpendicular to the monolayer showed that viral budding was highly asymmetric, with 88% of VSV emerging from the basolateral surface, and 94% of influenza viruses emerging from the apical surface. Since polarity of viral budding depends on the asymmetric distribution of newly-synthesized envelope glycoproteins, the results of our experiments show that islet cells have the capacity to sort out and address different surface proteins to specific plasma membrane domains; this suggests that the expression of endogenous membrane proteins, such as receptors, may be polarized.

326. Blood glucose self-monitoring and metabolic control in Type 1 (insulin-dependent) diabetes

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Home blood glucose monitoring may be useful to achieve better metabolic control in Type 1 diabetes. The aim of this study was to evaluate long-term results in a large population. We interviewed 282 conventionaly insulin-treated patients regularly attending our clinic (mean ± SD age 48 ± 16.6 years, mean duration of disease 14 ± 10.5 years, number of daily injections 1 = 45%, 2 = 50%). Home blood glucose monitoring was performed by 65% of the patients for 18.8 ± 13 months, 60% using a meter. 79% continued to monitor urines. The mean weekly number of blood glucose determinations was 14 ± 11, the number increasing with that of daily injections ($r = 0.51$, $p < 0.001$). Insulin self-adjustment was made by 69% of patients using home blood glucose monitoring. Mean ± SD HbA_1 at the time of the interview (kit Cordis, normal < 7%) was similar for patients monitoring blood only (9.3 ± 2.1%; $n = 40$), blood and urine (9.2 ± 2%; $n = 128$), urine only (9.3 ± 1.7%; $n = 81$), or without any surveillance (9.5–1.8%; $n = 22$). The occurrence of hypoglycaemic attacks during the last 3 months was not statistically different in the four groups. In conclusion, the influence of home blood glucose monitoring on metabolic control may be over-emphasized as currently performed by diabetic patients in everyday life.

327. Evidence for a rôle of calcium-phospholipid-dependent protein kinase in insulin secretion

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A change in cytosolic calcium is the major trigger for insulin secretion but the mechanism of its action is unknown. A potential target for calcium is calcium-phospholipid-dependent protein kinase (C-kinase). Experimental support for this hypothesis includes the demonstration of secretion-associated changes in inositol phospholipid turnover and the stimulatory effect on insulin secretion of 12-0-tetradecanoylphorbol 13-acetate (TPA), a known activator of C-kinase. We have previously identified and characterised C-kinase from islets and cloned (HIT-T15) hamster β-cells. In the present study we have used the purified β-cell enzyme to compare the effects of various agents on C-kinase and on insulin secretion in isolated rat islets. TPA increased C-kinase activity at concentrations that also potentiated glucose-stimulated insulin secretion. Trifluoperazine (40 μmol/l) and trifluoperazine oxide (40 μmol/l) inhibited C-kinase activity by 50% and 26%, respectively, correlating with their relative effects on insulin secretion. Both diacylglyerol and TPA-activated C-kinase were sensitive to inhibition by retinal and retinol at concentrations of 1 μmol/l and 10 μmol/l, respectively, and in addition retinal 100 μmol/l produced a 45% reduction in glucose-stimulated insulin secretion. These studies give further support to the involvement of C-kinase in the insulin secretory process.

328. Outcome of infants of diabetic mothers in the United Kingdom

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Data on the 1034 diabetic subjects reported from 188 hospitals was presented at the 19th EASD Meeting. We report on the complex analysis of outcome, maternal diabetes and metabolic control. Infant mass correlated directly with maternal third trimester mean blood glucose for established and gestational diabetic patients. Degree of macrosomia was similar for both groups of diabetic infants but varied with gestational age reaching a maximum at 37 weeks. Second and third trimester maternal blood glucose correlated inversely with gestational age and gestation was longer for all White classes when maternal blood glucose was 6 mmol/l or less. Premature small and very large infants had a predicted increased perinatal mortality (> 16% and 10%, respectively). Pre-eclamptic toxaemia was more common in women with premature babies and in those with microangiopathic complications. Hospitals reporting 10–20 pregnancies were more likely to produce mature and less macrosomic infants but the largest units had the lowest incidence of neonatal hypoglycaemia. Surveillance was uniformly poor in the first trimester and a significantly greater malformation rate was predicted in infants born to women with no recorded first trimester blood glucose. Malformations accounted for half the neonatal deaths. The UK experience has shown that metabolic control is a major factor in determining outcome.

329. Somatostatin action on hypoglycaemia induced by tolbutamide

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The capacity of somatostatin to inhibit insulin secretion was used to study the hypoglycaemic action of tolbutamide. A tolbutamide test (1 g, intravenously) was performed in 6 healthy subjects (mean age 32 ± 7 years) on two different days. On day 1 tolbutamide alone was injected, on day 2, immediately before tolbutamide, a bolus (250 μg) of somatostatin was injected and then 500 μg more infused for 60 min. Blood samples were taken at −15, 0, 2, 5, 10, 20, 30, 60 min and glucose and IRI levels were assayed on plasma. When tolbutamide was injected alone, the insulin peak was at 5 min (78 ± 12 mU/l) and the lowest glucose level was reached at 30 min (35 ± 6% fall versus basal values). The infusion of somatostatin and tolbutamide did not cause significant insulin release, with the lowest glucose level occurring at 60 min (15 ± 8% fall versus basal values, $p < 0.05$). In conclusion, (1) somatostatin administration blocks the insulin secretion induced by tolbutamide, (2) however, tolbutamide retains its capacity almost unhindered to lower blood glucose levels even if this action is delayed and less evident.

330. Carbohydrate metabolism in normal women using oral contraceptives containing levonorgestrel or desogestrel: a 6 month prospective study

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This study compared blood glucose, plasma insulin and glucagon levels during oral glucose tolerance tests performed before and after 6 months use of three new oral contraceptives containing ethinylestradiol and low doses of 19-norprogestogens, levonorgestrel or desogestrel (DOG). Thirty-eight healthy women were allocated at random to Trigynon (triphasic with levonorgestrel, Ovidol (sequential with DOG) or Marvelon (monophasic with DOG). A slight deterioration of glucose tolerance was observed after all three oral contraceptives, the areas under the glucose curve increasing by 12% ($p < 0.05$) with Trigynon, by 7% (NS) with Ovidol and by 9% (NS) with Marvelon. These changes were associated with a consistent 22–25% reduction in the insulin response in all three groups. Trigynon did not modify fasting glucagon nor its decline after glucose, the latter being reduced in the two groups receiving DOG as progestogen. Erythrocyte insulin receptor levels were not significantly influenced by the use of any of the three oral contraceptives. Thus, these three new products produce only minor deterioration of glucose tolerance in healthy women, while insulin resistance often reported with previous oral contraceptives was not seen in this study since plasma insulin levels were decreased and erythrocyte insulin receptors were not affected.

331. Insulin-stimulated phosphorylation of human placenta membrane actin. Involvement of serine specific protein kinase activity

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The β-subunit (M_r 95,000) of the insulin receptor contains an insulin-stimulatable tyrosine specific protein kinase that catalyzes its autophosphorylation as well as phosphorylation of a number of exogenous proteins like histone, casein, and others. We have shown that purified placental insulin receptor preparations can also catalyze phosphate incorporation into exogenously added muscle actin. As actin is a regular membrane constituent of non-muscle cells, its phosphorylation may be of physiological significance in insulin action. We

have therefore studied [^{32}P] incorporation from [γ-^{32}P]ATP into proteins of unfractionated trixon X-100 solubilized placenta membranes. After SDS-PAGE and autoradiography there appeared a phosphoprotein corresponding to M_r 41,000 which was identified by specific immunoprecipitation as actin. Phosphorylation of this endogenous membrane actin was about twofold increased by insulin and involved only serine residues. In contrast, phosphoamino acid analysis of actin after phosphorylation by partially purified membrane preparations yielded exclusively phosphotyrosine. Our results indicate that (1) endogenous membrane actin is susceptible to insulin-stimulated phosphorylation, and (2) in the intact placenta membranes there appears to be a serine specific protein kinase(s) stimulatable by insulin, probably via the insulin receptor-associated tyrosine specific protein kinase.

332. Risk factors of severe hypoglycaemia in Type 1 (insulin-dependent) diabetes

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In 31 Type 1 diabetic patients (10 with autonomic neuropathy), duration of diabetes 1–31 years, hypoglycaemia was induced by infusion of 0.15 IU $\cdot$ kg$^{-1}\cdot$h^{-1} of insulin. Arbitrarily, the 10 patients who had the slowest increase in blood glucose 2 h after stopping the insulin infusion (group A: 2.1 ± 0.2 mmol/l) were compared with the 10 patients who had the fastest increase in blood glucose (group B: 5.9 ± 0.6 mmol/l). The glucagon and growth hormone responses were the same, whereas adrenaline (twofold, $p < 0.01$), noradrenaline (2.4-fold, $p<0.01$), and cortisol (two-fold, $p<0.01$) responses were greater in group A. Plasma free insulin was similar until 50 min after stopping the insulin infusion. Thereafter it was higher (twofold, $p< 0.05$) in group A, where a threefold greater amount of insulin binding antibodies was also found ($p<0.05$). Rates of recovery from hypoglycaemia and amounts of antibodies were inversely correlated ($p<0.02$). Prevalence of autonomic neuropathy and duration of disease did not differ. In conclusion, enhanced counter-regulatory hormone responses in the group with slow recovery from hypoglycaemia could not compensate for the hypoglycaemic effect of a concomitant higher plasma free insulin concentration. Insulin binding antibodies, acting as a depot of circulating insulin, may be a risk factor of severe hypoglycaemia.

333. Continuous gastrin/CKK-producing cell cultures established from a transplantable rat insulinoma

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A slow-growing variant of the X-ray-induced transplantable NEDH-rat insulinoman (RIN) was maintained for a period of 5 years by selective transplantation of small-sized tumours from severely hypoglycaemic animals. From one tumour it was possible to establish a series of continuous cultures in vitro, of which an insulin-secreting cell line MSL-G was established by twice-repeated cloning. When transplanted into NEDH-rats these cells caused hypoglycaemia. Immunohistochemical characterization of the tumour identified a high frequency of insulin-producing cells (95%). A few scattered cells were positive for glucagon, somatostatin or gastrin/CKK, but none for pancreatic polypeptide. A morphologically different variant of MSL-G with reduced insulin production was isolated. This new variant (MSL-G2) was characterized in each of three successive passages using an immunocytochemical staining procedure with antisera to a panel of pancreatic hormones. The frequency of insulin-positive cells was dramatically reduced to $<1\%$. Glucagon and somatostatin were present in 10–20% of the cells, but surprisingly a rapidly-increasing fraction of the cells stained strongly for gastrin/CKK ($>60\%$ in the latest passage). We conclude that cells from the transplantable RIN-tumour which can proliferate in vitro have maintained a considerable potential to differentiate.

334. Inhibition of fatty-acid oxidation and insulin release in rat pancreatic islet

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The pancreatic β-cell may represent a fuel-sensor organ, the release of insulin evoked by nutrient secretagogues being attributable to increased oxidation of exogenous and/or endogenous substrates. If so, inhibition of endogenous fatty-acid oxidation, which accounts for a fraction of basal O_2 uptake, should impair insulin release. Methyl palmoxirate (McN-3716, 0.1 mmol/l), an inhibitor of long-chain fatty-acid oxidation, suppressed the oxidation of exogenous (U-^{14}C)palmitate by rat islets, and inhibited $^{14}CO_2$ output from islets prelabelled with (U-^{14}C)palmitate. Methyl palmoxirate failed to affect D-(U-^{14}C)glucose oxidation, NH_4^+ production, or $^{14}CO_2$ output from islets prelabelled with L-(U-^{14}C)glutamine. Methyl palmoxirate inhibited insulin release evoked by D-glucose, D-glyceraldehyde, 2-ketoisocaproate, L-leucine, 2-aminobicyclo(2,2,1)heptane-2-carboxylate or 3-phenylpyruvate. However, methyl palmoxirate failed to affect insulin release when the oxidation of endogenous fatty acids was already suppressed, e.g. in the presence of pyruvate or L-glutamine. Thus, methyl palmoxirate did not affect the increment in insulin output evoked, in the presence of D-glucose, by pyruvate or the release of insulin provoked, in the presence of L-glutamine, by D-glucose or L-leucine. These findings support the view that insulin release evoked by nutrient secretagogues tightly depends on the overall rate of nutrient oxidation, including that of endogenous fatty acids.

335. Educational courses for diabetic patients: long-term persistence and reinforcement of learning

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Diabetic patients coming to our clinic are usually invited to follow a systematic 18-h course on diabetes. We have evaluated knowledge 3–4 years after the a first course. To reinforce and renew it, we invited on five occasions 67 insulin-dependent diabetic patients to a second course lasting 15 h and organized on the basis of 10 'guided' round-table discussions. Two questionnaires (31 general section questions + 11 insulin section questions) similar to those used for the first course were distributed alternatively before and after the second course. Results, expressed as percentage of total score, were statistically evaluated with the paired Student's t-test. The scores obtained before the second course (79% and 90% respectively for the two sections) were slightly lower than those obtained after the first course (87% and 94%) and still significantly greater than those obtained before the first course (72% and 78%). They were not significantly improved after the second course (86% and 94%). In conclusion, our first course appeared to improve persistently patients' knowledge. The second course did not significantly improve it further, partly because of the higher starting level, and partly because of the different teaching method used.

336. Biological activity of iodinated GIP as tested in vitro by binding to β-cell plasma membrane and stimulation of insulin release

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Pure natural porcine GIP was labelled by chloramine T procedure at a mean of 0.11–0.19 iodine atom/molecule. After high performance liquid chromatographic purification in two steps, on a Sep-pack C 8 cartridge and a micro Bondapak C 18 column, the peaks of iodinated GIP were tested in different biological systems. In the two major peaks A3 and B3, ^{125}I-GIP was found to be specifically bound by membranes from insulin-secreting hamster pancreatic tumours. Specific binding was found similar in each peak but non-specific binding was higher with the B3 than with the A3 peak ($p<0.01$, $n=12$). In the β-cell plasma membranes, the number of high affinity sites was 219 ± 9 fmol/mg protein and the dissociation constant $2.05\pm0.1\times10^{-9}$ mol/l ($n=8$). The ability of iodinated GIP to stimulate insulin release in vitro was tested using the perfused isolated rat pancreas at glucose concentration (6.6 mmol/l) and GIP concentration (1 nmol/l). Under these conditions, it was verified that peak A3 and peak B3 stimulate insulin release to an extent which is at least equal to that obtained with unlabelled GIP. The effect of GIP, native or labelled, accounts for a 6- to 15-fold increase of insulin release above the basal. In conclusion, purification of iodinated GIP on high performance liquid chromatography gives the possibility to obtain an iodinated GIP which retains its biological activity.

337. Disappearance and reappearance of islet cell antibodies in cyclosporin A treated Type 1 (insulin-dependent) diabetes

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The effect of cyclosporin A (CYA) on islet cell antibodies (ICA) and the influence of ICA on CYA-induced remission were studied in 64 Type 1 diabetic patients (mean age 14.5 years) from diagnosis through 1–12 months of CYA-treatment (group 1), and 55 Type 1 diabetic patients (aged < 30 years) were followed from diagnosis through 24 months of conventional insulin treatment (controls). The proportion of ICA-positive sera (with titres > 3) was 62% and 67% in group 1 and the controls, respectively. The frequency of ICA titres > 3 dropped more rapidly in group 1 than in controls (at 3, 6, and 9 months: $p = 0.13$, 0.025, 0.046). No CYA-treated patient had ICA after 12 months, versus 42% of controls. CYA was discontinued in 22 patients so far observed for 1–9 months. At CYA-withdrawal all were ICA-negative. Ten were ICA-positive at diagnosis. ICA reappeared in six on average after 3 months. In 12 negative at diagnosis, ICA appeared in two ($p = 0.043$) after CYA-withdrawal. Reappearance of ICA was associated with high titres at diagnosis. In conclusion, ICA did not influence CYA-induced remission; and ICA reappearance occurred in half of the patients quite rapidly after CYA-withdrawal, but at present it is unknown whether this predicts clinical relapse.

338. Cardiovascular responses to graded exercise and autonomic neuropathy in Type 1 (insulin-dependent) diabetic patients

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Aim of this study was to correlate the cardiovascular response to graded exercise (on ergometer cycle) with the presence of autonomic neuropathy (AN) in Type 1 diabetes. Ten diabetics with AN (mean age 39.9 years, deep breathing 9.1 ± 2.4 beats/min, one with ortostatic hypotension), 14 diabetics without AN (mean age 37.6 years, deep breathing 21.0 ± 5.5 beats/min) and 16 control patients (mean age 35.8 years, deep breathing 25.9 ± 4.7 beats/min) were examined. Results: resting heart rate (HR) and systolic blood pressure were significantly higher in diabetic patients with AN than in the other two groups. The increase in heart rate at lowest work load (50 W) was lower in diabetics with AN ($p < 0.001$) than in the other groups and significantly correlated with deep breathing ($r = 0.676$). Maximum heart rate in the same subjects was significantly lower (138 ± 16 beats/min) compared with diabetics without AN (157 ± 14 beats/min) and controls (168 ± 14 beats/min). Greatest tolerable workload was also reduced (87 W in diabetics with AN, 135 W in diabetics without AN and 160 W in controls) as was maximal oxygen uptake (maximum heart rate × maximum systolic blood pressure/1.000: 27 ± 4.5 in diabetics with AN, 30.9 ± 3.2 in diabetics without AN, 32.8 ± 4.6 in controls). It is concluded that the presence of autonomic neuropathy may be responsible for impaired cardiovascular responses to graded exercise in diabetic patients.

339. Glucose tolerance and microangiopathy: 7 years follow-up

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In 1977, 229 subjects with risk factors underwent a 2-h oral glucose tolerance test (GTT). Three (1.3%) showed fasting hyperglycaemia; the remainder underwent a second oral GTT 7 years later. First oral GTT: glycaemia 2 h after 100 g glucose ≤ 160 mg%: 177 subjects (77%). Second oral GTT: glycaemia ≤ 160: 144 subjects (81%), glycaemia > 160 ≤ 200: 22 subjects (12%), glycaemia > 200: 11 subjects (6%). First oral GTT, glycaemia > 160 ≤ 200: 34 subjects (15%). Second oral GTT, glycaemia ≤ 160: 17 subjects (50%), glycaemia > 160 ≤ 200: 10 subjects (29%), glycaemia > 200: 7 subjects (21%). First oral GTT, glycaemia > 200: 15 subjects (7%). Second oral GTT glycaemia ≤ 160: 7 subjects (47%), glycaemia > 160 ≤ 200: 5 subjects (33%), glycaemia > 200: 3 subjects (20%). The subjects who had glycaemia ≤ 160 at the second test presented microaneurysms at fundus oculi in 11%, exudates in 3%, haemorrhages in 1.2%. Six percent were Albustix positive. Those with glycaemia > 160 ≤ 200 had microaneurysms in 14%, exudates in 3%, haemorrhages in 5%, proteinuria in 5%. Finally those with glycaemia > 200 had microaneurysms in 10%, exudates in 5%, none had haemorrhages, 7% had proteinuria. Hypertension was present in 18% of patients. Improvement of glucose tolerance correlates with weight reduction and not with exclusion of simple sugars from diet. Presence of microangiopathy correlates with hypertension not with glycaemic values.

340. Metabolic consequences of continuous hyperinsulinaemia in normal man

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Hyperinsulinaemia exists in pathological states, such as maturity-onset diabetes and obesity. Hyperinsulinaemia may be primary or secondary to insulin resistance. The present study examined the metabolic effects of sustained hyperinsulinaemia-euglycaemia in normal subjects. Basally and 1 h after 20 h of hyperinsulinaemia (25 ± 0.6 mU/l) or saline, 18 subjects had assessments of (a) glucose turnover, using ^{3}H-3-glucose; (b) insulin sensitivity, using the hyperinsulinaemia (60 ± 2 mU/l)/euglycaemia (4.8 ± 0.02 mmol/l) clamp or the intravenous glucose tolerance test; (c) monocyte insulin receptor binding. Hepatic glucose production (R_a) was suppressed by > 95% during the euglycaemic clamp and remained so during the sustained hyperinsulinaemia; following cessation of insulin, R_a and glucose disposal returned to normal by 20 and 40 min respectively. Sustained (20 h) hyperinsulinaemia resulted in (1) reduced glucose utilisation (6.4 to 4.5 $mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.05$) and insulin action (0.043 to 0.03 per min, $p < 0.05$) during the 40–80 min phase of the euglycaemic clamp; and during the 80–120 min phase, glucose utilisation and insulin action were less but not significant; (2) reduced K values on intravenous glucose tolerance test (2.7–1.8, $p < 0.02$) and higher plasma glucose values ($p < 0.02$) from 40–180 min in the presence of similar or higher insulin levels; (3) monocyte insulin receptor binding was not altered. These studies indicate that hyperinsulinaemia may lead to decreased insulin action despite normal receptor binding.

341. Effect of glucose on ion movements and insulin release of islets of fetal, newborn and adult rats

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Fetal islets exhibit no or little insulin secretion when challenged with glucose. In adult rats glucose-induced insulin release is associated with inhibition of potassium permeability and the subsequent stimulation of calcium uptake. In collagenase-isolated islets of fetal, newborn (5 days) and adult rats the effect of glucose on ^{86}Rb efflux, lanthanum-non-displaceable ^{45}Ca uptake and insulin secretion was studied. In islets of adults glucose (5.6 mmol/l) inhibited the ^{86}Rb efflux almost maximally (3 mmol/1: 1.5%/min; 5.6 mmol/1: 0.99%/min) and was not further decreased at 16.7 mmol/l. In islets of fetal rats the efflux rate is maximally inhibited even in the presence of 3 mmol/l glucose (0.99%/min) and was not further diminished by 5.6 or 16.7 mmol/l. Increasing glucose concentrations caused a slight decrease of ^{86}Rb efflux rate in islets of newborn rats. In contrast to islets of adult and newborn rats in fetal islets 16.7 mmol/l glucose neither increased ^{45}Ca uptake (adult: 186 versus 453, newborn: 190 versus 283, fetus: 96 versus 91 fmol ^{45}Ca/20 islets per 30 min in the presence of glucose, 3 and 16.7 mmol/l) nor stimulated insulin secretion. The data suggest that the failure of fetal islets to exhibit insulin secretion in response to glucose may be due to the fact that increasing glucose concentrations neither change Rb efflux nor Ca uptake.

342. Islet cell antibodies predict loss of fasting C-peptide

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It is controversial whether the presence of islet cell cytoplasmic antibodies (ICA) correlates with residual β-cell function. Fasting C-peptide and ICA titres were therefore determined in 84 Type 1 (insulin-dependent) diabetic patients (30 females, 54 males, aged 8–69 years) and followed prospectively during the first 30 months of insulin treatment. At the time of diagnosis 57% had ICA. Only 32% remained positive for 30 months. However, their ICA titres declined from 1:27 (median; range 1:1–1:729) at diagnosis to 1:3 (median; range 1:1–1:243) after 30 months ($p < 0.01$). Those patients who became ICA-negative within 30 months had a titre of 1:3 (median; range 1:1–1:81) at diagnosis. Fasting C-peptide was maximal 1–3 months after diagnosis (median 22, range < 0.06–0.54 nmol/l) and did not differ between the ICA-negative patients and the 16 remaining ICA-positive for 3 months. The progressive decline in fasting C-peptide was, however, less in the ICA-negative patients, who had 0.15 nmol/l (median; range < 0.06–0.44) at 30 months, compared with 0.06 nmol/l (median; range < 0.06–0.17) in the ICA-positive patients ($p < 0.02$). It is concluded that the presence of ICA throughout the first 30 months of

Type 1 diabetes is associated with an accelerated decline of fasting C-peptide.

343. Reduced urinary kallikrein activity is associated with microangiopathy in diabetic patients

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Renal kallikrein, a tubular enzyme acting through kinin liberation, may participate in the control of renal circulation and blood pressure. To study whether an impairment of its secretion is associated with microangiopathy in diabetes, a cross-sectional study was performed on 30 control and 60 diabetic subjects with or without retinopathy. Urinary Kallikrein Activity (UKA) was determined in 24-h urine by kininogenase activity, with and without trypsin pre-incubation. Renal function was assessed by creatinine clearance and radioimmunoassay of albumin excretion. UKA was significantly lower in patients with maculopathy ($n = 8$) and proliferative retinopathy ($n = 8$) than in control subjects (23 ± 7 and 11 ± 2 versus 82 ± 10 μgLBK/ min, $p < 0.01$ and $p < 0.001$, respectively). It was not modified in patients without retinopathy or with background retinopathy. The ratio of inactive to active kallikrein, obtained from trypsin pre-incubation, was not different in diabetic or control subjects (2.09 ± 0.41 versus 1.71 ± 0.31; NS). Albumin excretion, which was higher in patients with maculopathy or proliferative retinopathy than in the other patients, was negatively related to log UKA ($r = 0.49$; $p < 0.005$). UKA was not related to creatinine clearance in any group. In conclusion, (1) UKA is reduced in diabetic patients with microangiopathy; (2) this is not corrected by trypsin activation; (3) impaired UKA is related to exaggerated albumin excretion.

344. The ultrastructural changes of the β-cell and the insulin secretion by islets from lactating and non-lactating rats

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Twenty days after delivery, the insulin content/islet is not significantly different between lactating (1.27 ± 0.19 mU/islet) and non-lactating rats (0.94 ± 0.11). Less insulin is released by islets from lactating rats compared with non-lactating rats, whether in the absence of exogenous nutrient (14.7 ± 2.4 versus 38.7 ± 5.7 μU/90 min per islet), in the presence of D-glucose (154.6 ± 7.7 versus 184.1 ± 8.6 μU/90 min per islet) or the association of L-leucine and L-glutamine (80.5 ± 4.7 versus 186.2 ± 15.6). The volume density of the light granules in the β-cells (2.59 ± 0.21 versus $4.76 \pm 0.32\%$) and of their secretory content (1.85 ± 0.15 versus $3.09 \pm 0.21\%$) are lower in lactating animals. The volume density of the dark granules (12.49 ± 0.76 versus $14.10 \pm 0.76\%$) and their secretory content (4.57 ± 0.30 versus $5.01 \pm 0.28\%$) are not different. The lower volume density of the light granules of lactating rats combined with a lower secretion capacity fits with the hypothesis that the light granules are associated with activated turnover of insulin, while the dark granules represent the insulin reserve. The evidence that the insulin content/islet is not reduced must be interpreted by the fact that the proportion of β-cells in the islets is not at variance. Moreover the volume density of the dark granules is not different between the two groups.

345. Ionic response to cholinergic agents in pancreatic islets

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A vagal stimulation of insulin release is thought to contribute to the response of the endocrine pancreas to food intake. In the presence of glucose, cholinergic agents indeed enhanced electrical activity and insulin release in the pancreatic β-cell. The ionic determinants of such a functional response are poorly understood. In perifused rat islets, carbamylcholine (0.01–1.0 mmol/l) provoked a dose-related stimulation of ^{45}Ca and ^{86}Rb outflow and insulin release. At low glucose concentration, the stimulation of both ^{45}Ca and insulin release displayed a biphasic pattern and was rapidly reversible. Such a stimulation was observed at low, intermediate and high glucose concentrations (5.6–16.7 mmol/l). The cationic response persisted in the absence of Ca^{2+} and presence of EGTA, but was suppressed by atropine. In the presence of glucose (8.3 mmol/l), carbamylcholine caused a transient decrease in effluent radioactivity from islets prelabelled with $^{32}PO_4^{3-}$, whereas a rise in glucose concentration provoked a typical phosphate flush. Thus, the cholinergic depolarization of the plasma membrane is apparently not attributable to a decreased K^+ permeability or increased phosphate outflow. The present results rather suggest that cholinergic agents mobilize Ca^{2+} from cellular sites, this effect persisting in the absence of extracellular Ca^{2+}.

346. No evidence of circulating immune complexes or humoral immuno reactivity to glomerular basement membrane in early diabetic nephropathy

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Fifty male Type 1 (insulin-dependent) diabetic patients with clinical nephropathy without renal failure and 20 diabetic subjects without clinical nephropathy comparable with regard to sex, age, and duration of diabetes were investigated to examine the significance of humoral autoimmunity in the pathogenesis of clinical diabetic nephropathy. The presence of antibasement membrane antibodies were investigated with four different antigen-preparations: guanidine-HCl extracted glomerular basement membrane, a Goodpasture antigen, the basement membrane component laminin, and the basement membranes in fixed sections of normal human tissue. Circulating immune complexes were determined by four different methods: solid phase $C1_q$ radioimmunoassay, solid phase conglutinin-binding assay, polyethylene glycol precipitation complement consumption method and surface bound IgG and IgM on red blood cells. Complement activation was investigated by measuring the C3d concentration in plasma. All diabetic patients with clinical nephropathy (except one) reacted negatively, when examined for immunoreactivity to glomerular basement membrane antigens. No difference in circulating immune complexes between diabetic patients with or without clinical nephropathy was observed; neither did we find evidence of an increased activation of the complement system in those with clinical nephropathy. We conclude that neither antibasement membrane antibodies nor circulating immune complexes play a major role in the pathogenesis of clinical nephropathy in patients with Type 1 diabetes.

347. Islet cell cytoplasmic and cell surface antibodies react with a human pancreatic β-cell clone

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A human pancreatic β-cell line (clone JHPI-1) was tested for reactivity with islet cell antibodies (ICA) and islet cell surface antibodies (ICSA) and compared with previously-established methods. Using indirect immunofluorescence tests, we found that JHPI-1 cells reacted with ICA and ICSA in sera from Type 1 (insulin-dependent) diabetic patients. In comparison with rat islet cells, the prevalence of ICSA reactive with JHPI-1 cells was increased. The binding of ^{125}I-protein A to JHPI-1 cells exposed to sera from diabetic patients correlated with but was greater than the binding to rat and/or mouse islet cells similarly treated. JHPI-1 cells reacted with ICA-IgG in 7 out of 10 Type 1 diabetic patients, this reactivity correlating with that determined on sections of frozen human pancreas. In conclusion, JPHI-1 cells derived from a human pancreas were used to detect ICSA and ICA. JHPI-1 cells tended to express greater species-specific reactivity for ICSA.

348. An unbiased, flexible computer program for glucose clamping, with graphics and running statistics

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Imperfect algorithms for glucose clamping lead to sub-optimal clamp conditions and may allow observer bias. We have developed a microcomputer BASIC program, with minimal physiological assumptions of glucose insulin interaction, to assist euglycaemic or hyperglycaemic clamping. The program calculates the increments or decrements of glucose concentration observed and relates these to the glucose infusion rate required to maintain the desired glucose concentration. Data are inserted into this array for each new reading made: conflicting data are averaged and the array maintained in a rational order. Changes of clamp level can be achieved easily. The program in automatic mode simply requires blood glucose values estimated at predetermined intervals and provides the predicted rate of glucose that should be infused. However the program utilizes any available data at any time: this allows for crises in blood sampling, infusate changes etc. Running statistics and current data are up-dated on a graphic screen display and the program informs one when the steady-state has been achieved, and of the steady-state glucose infusion rate. Graphics and statistics can be printed. This method of glucose clamping is easy

to use, unbiassed and allows maximum flexibility of data entry. We have developed Apple and CP/M versions.

349. The metabolic importance of even minimal β-cell secretion in insulin-treated diabetes

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The aim of this study was to evaluate the importance of residual β-cell function on glycaemic control in 131 insulin-treated diabetic patients. β-function was evaluated from plasma C-peptide (CP) 6 min after intravenous injection of 1 mg glucagon. For all patients CP correlated with HbA_1 ($r = 0.29$, $p < 0.001$). Dividing the patients into the groups CP < 0.06; 0.06–0.30; 0.30–0.60 and ⩾ 0.60 pmol/ml, HbA_1 (median) was higher in the group without β-cell function (11.9%, range 8.4–15.6%, $n = 51$) compared with each of the other groups (10.4%, range 8.3–14.2%, $n = 30$), (9.7%, range 8.1–16.6%, $n = 17$) and (10.1%, range 7.6–14.4%, $n = 32$) respectively ($p < 0.001$). No differences were found in HbA_1 between the groups with β-cell function. Patients without β-cell function were treated with more insulin/kg than patients in the groups with β-cell function ($p < 0.001$). HbA_1 was positively correlated with duration of diabetes, body mass index, insulin dose but negatively with age. Using a multivariate regression analyses 10% of the variation in HbA_1 was explained by CP ($p < 0.001$) and 3% by body mass index ($p < 0.05$), while age, duration of diabetes and insulin dose were of no importance. In conclusion, even minimal β-cell function is of metabolic importance in insulin-treated diabetes. Body mass index is of importance too, probably caused by the inverse correlation with insulin sensitivity.

350. Diabetic cardiomyopathy: an m-mode computerized echocardiographic study

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Twenty-six patients (aged 17–35 years) with Type 1 (insulin-dependent) diabetes for 3 years or more, without clinical heart disease, were studied for subclinical impairment of left ventricular function; patients were divided into those with (group 1) or without (group 2) retinopathy. The interval between minimum ventricular dimension (LVIDmin) and mitral valve opening (MVO) was increased ($p < 0.001$) compared with the control subjects (mean ± SD: group 1: 41.00 ± 12.29; group 2: 29.21 ± 12.99; controls: 10.20 ± 8.88). Furthermore, a close relation between the duration of diabetes and LVIDmin-MVO interval was found (*r:* 0.496; $p < 0.01$). The displacement ratio (%LVIDd) during LVIDmin-MVO interval was increased (mean ± SD: group 1: 12.43 ± 5.56 ($p < 0.001$); group 2: 8.21 ± 5.51 ($p < 0.02$); controls: 3.20 ± 3.43). This proves the existence of impairment of ventricular relaxation caused by incoordination between posterior wall and mitral valve movement due to the presence of wall stiffness. It is clear that there are often subclinical abnormalities resulting in impairment of diastolic function that correlate with the duration of diabetes and presence of retinopathy. A maximal stress test on the ergometric bicycle did not show any significant difference with the control subjects.

351. How far will Type 1 (insulin-dependent) diabetic patients change their eating habits?

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We have shown previously in a group of 40 poorly controlled Type 1 diabetic patients that imaginative dietary teaching can improve compliance and metabolic control, even when the dietary advice is simple. Patients were asked to keep the amount and distribution of carbohydrate consistent from day-to-day but could eat any type of food they wanted. The study was continued for a further 10 months to see whether any additional benefit could be obtained by asking a random group to change to a high fibre-carbohydrate/low fat diet (group HC/LF) compared to continuing their previous diet (group C). Knowledge and compliance remained high in both groups during follow-up. Fat intake fell in the HC/LF group compared to group C (33.9 ± 1.2% versus 42.0 ± 6.3%; $p < 0.02$) but carbohydrate, fibre and calorie intakes were no different between the groups. In addition, while HbA_1 remained low in group C during follow-up (final HbA_1 9.5 ± 0.4%), there was a rise in HbA_1 during the first 4 months in the HC/LF group (9.4 ± 0.5 to 11.2 ± 0.5%; $p < 0.01$) which fell over the next 6 months (10.0 ± 0.6%). Patients who are already well controlled on a simple diet derive no extra benefit from a high carbohydrate/low fat diet.

352. Insulin accelerates leucocyte cytosol vesicle motion

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Insulin is known to affect the biochemical behaviour of cells, but it is less widely appreciated that the hormone influences microscopically detectible cell organelle motion as well. Leucocytes were removed from the buffy coat of EDTA-anti-coagulated blood and resuspended in high potassium phosphate-buffered saline containing 0.1% bovine albumin. The effect of 0.15–15 mU/l insulin on leucocyte behaviour was observed using differential interference contrast (Nomarski) optics and an inverted video microscopy system. Time delay videotaping was utilized to enhance motion recognition. Activity of small (< 100 nm) organelles near the cell surface in areas away from pseudopod formation in motile cells and in irregularly distributed areas in non-motile cells was markedly accelerated by insulin. Insulin strikingly increased the number of evanescent shadows generated by intracellular organelles near the cell surface. Insulin markedly raised the number and/or velocity of these surface adjacent particles, suggesting that cell vesicle to membrane fusion is accelerated. While the mechanism for this effect of insulin is not clear, increased cell organelle motion could certainly contribute to or modify a number of metabolic processes and should be taken into account in our perception of insulin action.

353. Identification of islet cells displaying glucose-induced electrical activity

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Electrical signals recorded in intact islets have been hitherto attributed to βcells whenever they were glucose-induced, even though impalements were often performed at the heterocellular islet periphery where β and non-βcells are coupled. We have now identified the cells penetrated in mouse islets, using electrodes filled with 4% Lucifer Yellow in LiCl. Following recording of glucose (> 11.1 mmol/l)-induced electrical activity, Lucifer yellow was injected in monitored cells via the recording electrode, using hyperpolarizing pulses (0.1 nA, 0.5 Hz, 900 ms) for 2–20 min. Thereafter, the persistence of glucose-induced electrical activity and the intracellular location of the electrode were reassessed. The islets were then fixed in 4% paraformaldehyde, Epon embedded, and serially sectioned. The monitored cells, localized on sections by the green Lucifer yellow fluorescence, were identified by a red immunofluorescent staining with anti-islet hormones sera and rhodamine. In five islets, (three peripheral and two central impalements) a regular burst activity with superimposed spikes was recorded from individual βcells or groups of coupled βcells. In another peripherally-penetrated islet, a continuous spike activity was recorded from a coupling territory comprising α and βcells. Thus, glucose-induced electrical activity can be recorded both from homologous and heterologous groups of coupled islet cells.

354. Modulation of the glucose-induced electrical activity in single β-cells of the mouse pancreas by endogenous cyclic AMP

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In this study we investigated with micro-electrodes the effects of forskolin, an activator of the adenylate cyclase, on the biphasic pattern and on the slow cyclic variations of the glucose-induced electrical activity in single βcells. Stimulation with glucose produced a biphasic pattern of electrical activity corresponding to the biphasic insulin release. Pre-treatment with forskolin (1 μmol/l) for 5 or 10 min did not accelerate the appearence of electrical activity in response to stimulation with 10 mmol/l glucose. However, it markedly intensified the first phase of electrical activity which is assumed to be mainly due to a calcium inward current. Simultaneously the first phase of insulin release was considerably increased. Stimulation with 15 mmol/l glucose showed (under steady state in about 50% of the βcells) slow cyclic variations of the intensity of electrical activity which were superimposed on the periodic slow waves and intervals. These slow cyclic variations were not altered by blockade of cholinergic or adrenergic receptors. On the contrary, forskolin (0.2 μmol/l) reversibly abolished these slow oscillations and produced a regular slow wave pattern. The data show that elevation of intracellular cyclic AMP levels modulates

the membrane properties of βcells presumably by acting on the gating mechanism of the calcium channel.

355. Thiourea derivatives acutely potentiate glucose-induced insulin secretion in vitro and in vivo: augmentation of glucose elimination by propylthioracil

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Chronic administration of propylthiouracil (PTU) to rats has been shown to be associated with increased insulin secretory response from their perfused pancreases when challenged with glucose. To investigate whether thiourea derivatives directly act on βcells, the effect of PTU and related compounds, such as thiourea, thiamazole and methylthiouracil on insulin secretion were studied in collagenase-isolated rat pancreatic islets. Additionally, the effect of a single oral dose of PTU (50, 100 and 200 mg/kg) on plasma insulin and glucose was tested in anaesthetized rats. In the presence of glucose (2.8 mmol/l) none of the tested compounds stimulated the discharge of insulin from pancreatic islets. All of the above substances, however, augmented insulin releasing capacity of glucose (11.1 mmol/l) in a dose-related manner – PTU being the most potent agent. In vivo PTU (100 and 200 mg/kg) significantly augmented insulin secretion in response to an intravenous glucose load (0.5 g/kg). Accordingly, the rate constant of glucose elimination (K) was increased (3.75 versus 5.40* versus 5.52**; $*p < 0.02$, $**p < 0.01$). The data suggest that thiourea derivatives acutely sensitize the βcell to the insulin triggering action of glucose. Further elevation of plasma insulin levels by PTU during an intravenous glucose tolerance test possibly leads to the increase of glucose elimination rate.

356. Comparative contribution of glucose, fructose and galactose during acute stimulation of glycogenesis by insulin in cultured fetal hepatocytes

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Hepatocytes transplanted from 18 day-old rat fetuses, when grown for 2 days in the presence of cortisol, respond to insulin by increased glycogen synthesis. The influence of fructose or galactose supplementation (0.1–5 mmol/l) to the medium containing 4 mmol/l glucose was tested on basal and insulin-stimulated glycogenesis, using separate incorporation of any of the ^{14}C-labelled sugars into glycogen for 2 h. A competition of unlabelled fructose (5 mmol/l) with ^{14}C-glucose was observed (20%), which was more pronounced in the presence of insulin (35%), whereas galactose, for concentrations as low as 0.5 mmol/l, displaced ^{14}C-glucose efficiently. A maximal effect was obtained with 1 mmol/l galactose and concerned 40% and 55% of the total radioactivity incorporated in the absence and presence of insulin, respectively. When measured with ^{14}C-fructose or ^{14}C-galactose, the glycogenesis was stimulated by insulin only three- or twofold, respectively, i. e. much less than with ^{14}C-glucose (fivefold). Incorporation of either ^{14}C-fructose or ^{14}C-galactose together with ^{14}C-glucose were additive. This revealed a clear glycogenic effect of galactose (0.5–5 mmol/l), especially in the absence of insulin and a poor one with fructose (2–5 mmol/l) only detected on basal glycogenesis. These results suggest that different pathways can be modified by insulin, excluding a stimulation uniquely at the level of the terminal glycogenosynthetic pathway.

357. Diagnosis of subclinical retinopathy, peripheral and autonomic neuropathy in Type 1 (insulin-dependent) diabetic adolescents

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55 Type 1 diabetic adolescents (mean age: 15 years) with duration of disease > 5 years (mean: 9 years) were investigated for subclinical retinal, peripheral (N) and cardiac autonomic (C) nervous system impairment by fluorescein angiography, peripheral motor and sensory nerve conduction measurement by electroneurography, and heart rate variation test. Background or proliferative retinopathy was present in eight (14%), N in 16 (29%), C in 12 (22%) subjects; only four (8%) had alteration of all parameters. In patients with retinopathy but not in those with N or C, there was a significant increase of chronological age, duration of disease, stable HbA_1, total cholesterol and tryglicerides. Mean conduction velocities in all nerves tested and mean ratio of heart rate variation tests were significantly reduced versus normal subjects. Nerve conduction velocity showed a significant inverse correlation with duration of disease in median, ulnar and sural nerves, with chronological age in ulnar nerve and with HbA_1 in sural nerve only. A significant inverse correlation with duration of disease was found for Valsalva ratio only. N is seen alone in 6 out of 16 subjects and mean conduction velocities are significantly reduced when retinopathy and C are present: therefore electroneurography appears to be a valuable technique for early diagnosis of diabetic microangiopathy.

358. Stability of high and low glycosylated haemoglobin levels during a 3 year follow-up period of subjects with normal tolerance: the Israel GOH study

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In a representative sample of the Israeli Jewish population (aged 40–70 years ($n = 1058$) without known diabetes) examined for oral glucose tolerance and HbA_1, we found that while HbA_1 increased with increasing glucose intolerance ($p < 0.0001$), there was extensive overlap even between normal tolerance and newly diagnosed diabetes. Moreover 6% of those with normal tolerance (by National Diabetes Data Group criteria) had $HbA_1 \geq 8.5\%$ of total haemoglobin – a level considered to indicate unsatisfactory diabetes control. To evaluate the hypotheses that each subject has his own norm of HbA_1, we retested after a mean interval of 3 years subjects with initially normal tolerance (half with $HbA_1 \geq 8.0\%$ and half $< 6.5\%$). In all, 90% of subjects remained in their initial HbA_1 range. We conclude: (1) there are widely divergent individual norms of HbA_1, (2) monitoring the degree of control by HbA_1 should be individualized according to his own norm, (3) these stable norms may indicate either a stable dietary pattern or a genetically determined affinity for glycosylation level of proteins.

359. Association between islet cell antibodies, complement-dependent antibody mediated cytotoxicity, HLA antigens and residual β-cell function in newly diagnosed diabetes

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To demonstrate that anti-pancreatic cytotoxicity in vitro of sera from newly diagnosed diabetic patients is more related to residual β-cell function than to prevalence of islet cell antibodies, a study was performed in 71 Type 1 (insulin-dependent) and nine Type 2 (non-insulin-dependent) diabetic patients (mean age: 22 years, 37 females, 43 males). The metabolic-hormonal characterization was based upon estimations of blood glucose, HbA_1, serum ketone bodies, triglycerides, cholesterol and C-peptide in the fasting state and after glucose-glucagon stimulation. Islet cell surface (ICSA) and cytoplasmic islet antibodies (ICA) were determined by indirect immunofluorescence. The presence of complement-dependent antibody-mediated cytotoxicity (CAMC) towards neonatal rat islet cells of diabetic serum was tested using the ^{51}Cr-release assay. HLA typing (A-, B-, C-, DR locus) was performed employing the standard lymphocytotoxicity technique. Results: prevalence of ICA (59% in Type 1; 25% in Type 2 diabetic patients) or ICSA (68% in Type 1; 77% in Type 2 diabetic patients) was neither correlated to the residual β-cell function nor to the existence of CAMC (75%) and HLA phenotype, respectively. Diabetic patients without CAMC were characterized by an older age, higher C-peptide secretion, a low infection frequency before diagnosis. In conclusion, the process of β-cell destruction seems to be more reflected by CAMC than by ICA.

360. Familial diabetes and risk factors for cardiovascular disease in non-diabetic employees at the National Telephone Company, Lombardia

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The effects of first-degree familial diabetes on cardiovascular disease (CVD) risk factors were evaluated in 4989 non-diabetic employees of the National Telephone Company. 759 subjects (476 males, 283 females) reported familial diabetes (F+: positive, F−: negative history). Body mass index (BMI), fasting, first and second hour post-load glucose and cholesterol were higher in F+ subjects versus age and sex-matched F− ($p < 0.001$). In different age groups (< 30, 31–40, 41–50, > 50 years), the effect of familial diabetes was evaluated by sex

stratification. In F+ males this effect was stronger in younger groups who showed body mass index, systolic and diastolic pressures, cholesterol, triglycerides and basal, 1 h and 2 h blood glucoselevels higher than F− ($p < 0.002$). These phenomena declined with age disappearing over 50 years. In females, the effect of familial diabetes was weaker and delayed appearing after the age 30 years for body mass index and 1 h blood glucose value. In conclusion, our data suggest that first degree family history of diabetes in non-diabetic males carries higher values of fasting, 1 h and 2 h blood glucose and of several other risk factors for CVD, particularly in the younger age groups. In females, the phenomenon is delayed and less evident. This may indicate a multifactorial genetic link leading to increased CVD morbidity.

361. Dihomogammalinolenic acid inhibits platelet aggregation and stimulates platelet prostaglandin production in healthy subjects: absence of effect in insulin-dependent diabetes

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The effect of dihomogammalinolenic acid (DHLA) administration on platelet aggregation and prostaglandin production, erythrocyte fatty acid composition and serum lipids in healthy subjects and Type 1 (insulin-dependent) diabetic patients was compared. In healthy subjects, DHLA caused a significant inhibition of ADP-induced platelet aggregation and an increase in platelet prostaglandin$_1$ (PGE_1) release; Type 1 diabetic patients did not show these changes. Following DHLA, the diabetic patients did not show an increase in arachidonic acid content of erythrocytes as observed in healthy subjects: there were no differences, however, in thromboxane A_2 (TXA_2) or PGE_2 release between healthy and diabetic subjects before or after DHLA. DHLA induced a significant fall in serum non-esterified fatty acid concentrations in both groups without altering either cholesterol or triglyceride concentrations. These data show that (1) platelets from Type 1 diabetic patients may have a specific defect of PGE_1 synthesis quite distinct from the Δ^5 and Δ^6 desaturase defects known to be associated with experimental diabetes; this defect may contribute to platelet hyperaggregability in diabetes; and (2) DHLA has a potent antilipolytic effect in vivo.

362. Hyperchloraemic acidosis associated with insulin treatment of diabetic ketoacidosis

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The hyperchloraemic metabolic acidosis (HCMA) that occurs during the treatment of diabetic ketoacidosis has been attributed to loss of potential bicarbonate due to ketonuria, and has been said to exist prior to treatment. To determine whether HCMA can be induced acutely, five Type 1 (insulin-dependent) diabetic patients were maintained euglycaemic overnight with intravenous insulin using the Biostator. Insulin was discontinued for 15 h, then re-infused at 8 U/h for 10 h; subjects were infused with 0.9% saline throughout and had free access to water. Plasma ketone bodies increased from 1 to 9 mmol/l, then returned to 1 mmol/l with insulin re-infusion. pH (7.40 ± 0.01 to 7.30 ± 0.04) and HCO_3- (24 ± 1 to 15 ± 2) decreased ($p < 0.05$), then increased to 7.40 ± 0.01 and 23 ± 1 mmol/l respectively after insulin infusion. Chloride did not change during insulin withdrawal (104 ± 0.1 versus 101 ± 1 mmol/l, NS), but increased significantly during insulin infusion to 110 ± 1 mmol/l ($p < 0.05$). Serum sodium did not change, but potassium increased ($p < 0.05$) during insulin withdrawal, then returned to basal levels during re-infusion. In conclucsion, insulin withdrawal in Type 1 diabetes led to the development of ketoacidosis without accompanying hyperchloraemia. However, significant hyperchloraemia occurred following insulin therapy, consistent with recent studies in dogs demonstrating a renal tubular acidification defect induced by pharmacological doses of insulin.

363. Fasting plasma glucose – a poor detector of asymptomatic diabetes: The Israel GOH study

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New criteria adopted by the National Diabetes Group (NDDG) and World Health Organisate (WHO) define as diabetes subjects without fasting hyperglycaemia if blood glucose levels at the second hour post oral glucose load are ≥ 11.1 mmol/l. The NDDG criteria require also a 1 h level at ≥ 11.1 mmol/l. Normoglycaemia and hyperglycaemia are defined as fasting plasma glucose < 6.4 and ≥ 7.8, respectively. We assessed the distribution of fasting plasma glucose in asymptomatic subjects with no prior knowledge of the disease found to be diabetic by this definition in a study of a nationwide representative sample of the Israeli population aged 40–70 years ($n = 2387$) screened by oral glucose tolerance test (fasting, 1 and 2 h levels). Fasting plasma glucose (mmol/l) in 182 diabetic patients by WHO criteria was < 5.6 in 8%, 5.6–6.3 in 26%, 6.4–7.7 in 31% and ≥ 7.8 in 34%. Results were similar in 171 patients, diabetic by the NDDG criteria. No significant age, sex or ethnic differences were noted in this respect. We conclude that if follow-up and/or some form of treatment (dietary or otherwise) are deemed desirable for asymptomatic diabetics, the test of choice should be an oral glucose tolerance test.

364. Predicting diabetic nephropathy in insulin-dependent patients

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The aim of the study was to define the predictive value of raised urinary albumin excretion (UAE) for diabetic nephropathy in patients without clinical proteinuria. The role of abnormalities in glomerular filtration rate (GFR), renal plasma flow and blood pressure was also assessed. We re-examined all male diabetic patients examined in 1969–1976, fulfilling the following criteria: age at diagnosis < 20 years, initial duration 7–20 years, follow-up years > 7 years, and no proteinuria. Of 44 patients, 43 accepted re-examination. Patients with UAE at baseline of > 15 µg/min clearly progressed (12 out of 14) in contrast to patients with UAE < 15 µg/min where none of 29 patients progressed to diabetic neuropathy ($2p < 0.001$). Four developed raised UAE but no proteinuria. Patients showing progression and with UAE initially < 70 µg/min exhibited significantly raised GFR but normal renal plasma flow. Blood pressure was also significantly increased initially in progressive patients. Only one of the 29 patients with initial normal UAE developed proliferative retinopathy. In patients with raised UAE, nine out of 14 developed proliferations. We conclude that raised UAE ('microalbuminuria') strongly predicts diabetic neuropathy. Raised GFR and blood pressure may also be involved in the process leading to diabetic nephropathy. In addition raised UAE is predictive of proliferative retinopathy.

365. Tropical pancreatic diabetes in South India: heterogeneity in clinical and biochemical profile

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Tropical pancreatic diabetes (TPD) secondary to chronic calcific pancreatitis (non-alcoholic) is common in South India. Detailed clinical and biochemical studies were carried out in 35 TPD patients (24 males and 11 females). The mean age at onset was 26 years and mean duration of diabetes 6 years. Two patients had impaired glucose tolerance and the rest had overt diabetes. Malnutrition was present in 25% of patients. Mean ± SEM serum cholesterol levels were low: 4.03 ± 0.11 mmol/l in males and 4.26 ± 0.15 mmol/l in females. Serum C-peptide immunoreactivity (CPR) levels were highest in those responding to oral drugs (fasting, 0.17 ± 0.04; post-prandial, 0.48 ± 0.16 pmol/ml). The subgroup who were insulin-requiring but ketosis-resistant had lower CPR (0.13 ± 0.06 and 0.23 ± 0.05 pmol/ml). A small subgroup, who were ketosis prone, had negligible CPR (0.07 ± 0.01 and 0.09 ± 0.04 pmol/ml). Microangiopathy was common. Retinopathy occurred in 39% (background 32% and proliferative 7%), neuropathy in 32%, nephropathy in 11% and renal insufficiency in 6% of patients. Macroangiopathy was infrequent; ischaemic heart disease 3% and none had peripheral vascular disease. TPD in South India is heterogenous with regard to level of nutrition, severity of glucose intolerance, β-cell function, response to therapy and occurrence of microvascular complications.

366. ELISA (enzyme-linked immunosorbent assay) system for the detection of anti-insulin antibodies

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The current availability of porcine, beef and human insulin preparations raises the question of choosing the right one for each patient, specially when anti-insulin antibodies are present. Methods: Commercially-available preparations were used for coating the micortitre plates (Dynatech). The ideal concentration of insulin and serum dilution were elicited by checkerboard titration. All steps – coating, and additions of patient's serum, anti-human-IgG conjugated with alkaline phosphatase, and substrate – were carried out at 37 °C for 60 min. Only patients with known anti-insulin antibodies and a normal control group were tested. Results: ELISA results correlated well with the

method previously used. Patients with anti-porcine-insulin antibodies reacted also to a lesser extent with beef and human preparations (cross-reactivity 27.3 and 29–40% respectively). In conclusion, a fast and simple method giving reproducible results has been developed. It allows a clear-cut discrimination between positive and negative cases. The coating of various insulin preparations to one single plate eliminates inter-assay variation, while at the same time it provides valuable information about antigenic similarities of the used substances.

367. Chlorpropamide stimulates glycolysis and inhibits gluconeogenesis in isolated rat hepatocytes by increasing fructose 2,6-bisphosphate concentration

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Reduction of hepatic glucose output appears to play a relevant role in the improved glycaemic control elicited by prolonged sulphonylurea therapy. However, the biochemical basis of this hepatic action remains controversial. In this study we have studied in hepatocytes isolated from fed rats the effect of chlorpropamide on both glycolysis and gluconeogenesis, as well as on the cellular concentration of fructose 2,6-bisphosphate, a recently identified metabolite which controls the rate of the above-mentioned pathways. Chlorpropamide (1 mmol/l) was used, a concentration corresponding to the upper range of serum levels found in diabetic patients treated with the drug. The addition of chlorpropamide to hepatocytes incubated in the presence of glucose (10 mmol/l) provoked an increase of fructose 2,6-bisphosphate concentration (25–35% over the basal value), which persisted throughout the incubation time. Simultaneously, the rate of lactate production was significantly increased (53.8 ± 3.5 versus 39.7 ± 1.3 μmol/g of cells × 40 min). In correlation with these findings, chlorpropamide decreased the basal rate of gluconeogenesis (0.84 ± 3.5 versus 1.16 ± 0.12 μmol of (^{14}C)pyruvate converted into glucose/g of cells × 20 min). Our results indicate that chlorpropamide reduces hepatic glucose output by increasing fructose 2,6-bisophosphate concentration.

368. Rat liver nuclei contain active insulin receptors

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Insulin induces in its target cells a complex array of cellular responses, some of which appear to require transcription or replication of DNA. Nuclear insulin receptors have been suggested as playing a role in the generation of such insulin effects. To approach the potential biological role of nuclear insulin receptors, we examined their structure and function. For study of the insulin binding site, highly purified nuclei or glycoproteins derived from these nuclei were labelled with ^{125}I-photoreactive insulin. Analysis by SDS-polyacrylamide gel electrophoresis revealed the presence of a typical insulin binding subunit (α) with M_r 130,000. To search for putative β-subunits, glycoproteins obtained from highly purified nuclei were labelled with (γ-^{32}P)ATP, and analysed by SDS-polyacrylamide gel electrophoresis. Insulin increased several-fold the labelling of a single phosphoprotein with M_r 95,000, which was immunoprecipitated with specific antibodies against insulin receptors. Finally, nuclear insulin receptors catalysed the phosphorylation of exogenous histone. In conclusion, liver nuclei insulin receptors bind insulin (α-subunits), and display insulin-stimulatable protein kinase activity (β-subunits). It is possible that this functional insulin receptor kinase-complex is involved in the generation of late insulin effects through phosphorylation of nuclear proteins.

369. Effect of mellitin on insulin secretion from rat islets of Langerhans

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Mellitin is a hydrophobic polypeptide of 26 amino-acids which interacts with the plasma membrane of animal cells, altering their properties. Recent studies have shown that mellitin stimulates the release of prolactin from anterior pituitary cells, possibly by activating phospholipase A_2. Since phospholipase A_2 activation has been suggested to play a role in glucose-stimulated insulin secretion, we have investigated the effects of mellitin on insulin secretion from rat islets of Langerhans. Insulin secretion was stimulated by mellitin in a concentration-dependent manner with half the maximal response being obtained at a concentration of 4 mg/l. The secretory response to mellitin occurred rapidly within 1 min when islets were perifused with the agent, and was reversed on removal of mellitin. Enhancement of insulin secretion by mellitin was observed in the presence of sub-stimulatory (4 mmol/l) and sub-maximal (8 mmol/l) glucose concentration. Insulin secretion in response to mellitin was not inhibited by adrenaline (2 μmol/l) although removal of calcium from the incubation medium abolished the secretory response. These results will be discussed in relation to the possible involvmenet of phospholipase A_2 activation in the regulation of the insulin secretory process.

370. Childhood diabetes: diabetic control and psychological disturbance

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Forty-eight insulin-dependent diabetic children (aged 6–16 years) were studied to assess diabetic control and psychological status. Overall control was poor (mean ± SD HbA_1 $11.9 \pm 2.0\%$), insulin dosage was high (mean ± SD, 1.01 ± 0.35 units/kg) and single injections were common (65%). Psychological disturbance was present in 20% according to standardized questionnaires; psychiatric assessment revealed severe disorder in 6%. Associations between control, psychiatric status, demographic and clinical variables were examined using analysis of variance, Pearson product-moment correlations and non-parametric tests. Better diabetic control, assessed by HbA_1, was associated with higher insulin dose ($p < 0.05$). No association was found between HbA_1 and number of injections, C-peptide secretion, hospital admissions or insulin-antibody titre. A cluster of a relatively small number of emotional and behavioural features accounted for over 65% of the variation in diabetic control. Psychological disturbance was consistently associated with low HbA_1 (parent questionnaire $p < 0.05$; child questionnaire $p < 0.005$; psychiatridc assessment $p < 0.05$). Worry by the child about hypoglycamia was associated with low HbA_1 ($p < 0.025$). Good control was found in children whose parents warned about the ill-effects of not adhering to diet ($p < 0.025$), or who shared the tasks of giving injections ($p < 0.005$), and urine testing ($p < 0.01$).

371. Studies on the mechanism of inhibition of insulin release by noradrenaline

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Incubation of rat islets of Langerhans with 20 mmol/l glucose resulted in a large increase in insulin secretion, which was inhibited by noradrenaline. Inhibition was dose-dependent with half the maximal response at 10^{-7} mol/l noradrenaline and complete suppression of the glucose effect at 4×10^{-6} mol/l. Inhibition was relieved by yohimbine in a dose-dependent manner, but was largely unaffected by prazosin (up to 10^{-5} mol/l) suggesting mediation by α_2-adrenergic receptors. Noradrenaline also inhibited glucose stimulated ^{45}Ca accumulation in islet cells, although half-maximal inhibition required 8×10^{-6} mol/l. It is evident, therefore, that in the presence of noradrenaline there is a marked dissociation between the extent of islet cell Ca^{2+} uptake and the rate of insulin secretion. α-agonists have been shown to lower islet cyclic AMP levels. However, dibutyryl cyclic AMP (10^{-3} mol/l) failed to prevent noradrenaline-induced inhibition of insulin secretion. In contrast, dibutyryl cyclic AMP abolished the capacity of noradrenaline to inhibit glucose-induced $^{45}Ca^{2+}$-accumulation. These observations suggest that while noradrenaline can inhibit islet cell $^{45}Ca^{2+}$-influx, this effect may be secondary to lowered cyclic AMP and that the primary action of noradrenaline to inhibit insulin secretion may occur at a site which lies distal to both cyclic AMP generation and Ca^{2+}-influx.

372. Time-dependent changes in plasma glucose levels and glycogen metabolizing enzyme after injection of insulin into suprachiasmatic nucleus of rat

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The effect of insulin injection (30 μU and 300 μU) into the suprachiasmatic nucleus (SCN) on plasma glucose concentration was studied in rats anaesthetized with pentobarbital which were maintained under 12 h light (02.00–14.00 h), 12 h dark (14.0–02.00 h) lighting conditions. Insulin injections resulted in decreased plasma glucose concentrations within 2 min during the light period (10.00 h), but induced an increase within 2 min during the dark period (17.00 h). Plasma insulin levels changed in parallel with the plasma glucose levels after insulin injections. The insulin effects were abolished by the destruction of the SCN and intraperitoneal injection of hexamethonium (50 mg/kg), respectively. Furthermore, insulin injections induced a reduction in glycogen phosphorylase (GP) activity and an elevation in glycogen syn-

thetase activity in the liver during the light period, while they elicited a slight elevation in glycogen phosphorylase activity and a reduction in glycogen synthetase activity in the liver during the dark period. These findings suggest that insulin photoperiodically participate in the control of glucose homeostasis.

373. Cellular hypersensitivity to human pancreatic β-cell clone in diabetes mellitus and its relationship to the presence of islet cell antibodies

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Leucocyte migration tests against the human pancreatic β-cell clone (JHPI-1) were performed in 11 Type 1 (insulin-dependent) diabetic patients with islet cell cytoplasmic antibody (ICA) and/or islet cell surface antibody (ICSA), 13 Type 1 diabetic patients without ICA and ICSA, 14 Type 2 (non-insulin-dependent) diabetic patients diagnosed before the age of 25 years, 20 Type 2 diabetic patients diagnosed after the age of 25 years and 16 control subjects. The mean migration index values in each group were 85.5 ± 7.7%, 91.5 ± 9.9%, 98.3 ± 8.9%, 98.3 ± 7.4% and 100.0 ± 8.8%. The migration index values were more significantly decreased in the Type 1 than in the Type 2 diabetic patients and control subjects whether or not there were ICA and/or ICSA present in the patients' sera. Our data suggest that the lymphocytes of Type 1 diabetic patients may be sensitized by the pancreatic β-cell, which promoted leucocyte migration inhibition against JHPI-1. The ICA tend to disappear gradually after onset of diabetes while the sensitized lymphocytes to specific antigen were persistently present for a long time. Therefore, from the standpoint of immunological diagnosis, leucocyte migration tests against human pancreatic β-cells seem to be more useful than the detection of ICA in patients with long-standing Type 1 diabetes.

374. Non-invasive tracer method for the measurement of post-hepatic insulin appearance and its hepatic extraction in dogs

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The rate of post-hepatic insulin appearance (Ra) was determined using a constant infusion of [B1-H^3-Phe]-insulin and two-compartment analysis. To verify the calculations, unlabelled insulin was infused into four overnight-fasted dogs at continuously variable rates up to 250 mU/min. Somatostatin ($0.6\,ng \cdot kg^{-1} \cdot min^{-1}$) was infused to suppress endogenous insulin production. Normoglycaemia was maintained with a glucose infusion. Plasma levels of immunoreactive (IRI) and ^{3}H-insulin C were measured after initial purification on a C-18 column. IRI varied between 5 and 930 mU/l. Over this range the fractional disappearance rate of insulin (k) changed from basal (0.198 ± 0.40 per min) to a minimum of 54.5% below basal. The regression line for k as percentage of basal was $94.8 - 0.0625 \times IRI$, under these non-steady conditions. The system is therefore highly non-linear. To assess the matching of the calculated Ra to the rate of insulin infusions, the absolute value of the area between the curves was determined as a fraction of the area under the infusion curve and was 10.5 ± 2.2%. Our previous work showed that pre-hepatic insulin secretion could be calculated to the same degree of accuracy using C-peptide levels and mathematical deconvolution. In conclusion, both post- and pre-hepatic insulin secretion can be calculated within a 10% error. Subtraction yields a continuous non-invasive determination of the first-pass hepatic uptake of insulin.

375. Forskolin, islet cyclic AMP and insulin release: in vivo stimulation of insulin secretion by forskolin does not improve glucose elimination

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In rat islets the effect of forskolin (0.1–500 μmol/l) on cyclic AMP levels was measured. In addition the effect of forskolin (10 μmol/l) on insulin release was estimated in perifused islets. In fasted rats the effect of forskolin on serum insulin and glucose has been determined without and after an intravenous glucose bolus of 0.5 g/kg body weight. In incubated islets forskolin increased cyclic AMP concentration dependently, the ED_{50} being approximately (25 μmol/l). The maximal effect occurred after 5 min. In the presence of glucose (2.8 mmol/l) and forskolin (10 μmol/l) did not stimulate insulin release; however it potentiated both phases of 11.1 mmol/l glucose-induced insulin secretion. Forskolin (1.5 mg/kg) significantly increased blood glucose levels, which was associated with signficant elevation of serum insulin. During an intravenous glucose tolerance test forskolin (1.5 and 0.15 mg/kg) potentiated the insulin releasing capacity of glucose but no significant effect on glucose elimination was observed. From our data it is concluded that an increase of cyclic AMP induced by forskolin does not initiate insulin release but acts synergistically with the insulin-releasing effect of glucose. Since forskolin did not improve glucose tolerance it appears to counteract the blood glucose lowering effect of insulin directly or indirectly.

376. Pharmacokinetics and bioavailability of injected glucagon: differences between subcutaneous, intramuscular and intravenous administration

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The injection of glucagon by relatives in cases of severe hypoglycaemia is an integral part of modern diabetes teaching programmes. There is however uncertainty about the most efficient and practicable route of its administration. Six non-diabetic men were randomly assigned to the following experiments: 90 min after subcutaneous (SC) injection of 10 U regular insulin, 1 mg glucagon was administered (1) SC, (2) intramuscularly (IM), (3) intravenously (IV), or (4) no glucagon was given. Ten min thereafter, mean plasma glucagon levels had risen from 246 to 3233 ng/l (SC) and from 250 to 2638 ng/l (IM). In the IV experiment, at that time, mean glucagon values were 18033 ng/l. Accordingly, whether glucagon was applied SC or IM there was no difference in blood glucose behaviour (mean maximal rise at 25 min 2.15 mmol/l (SC) and 2.28 mmol/l (IM). The particularly high levels of plasma glucagon immediately after its IV injection were associated with a significantly steeper rise of glycaemia during the first 5 min, but the maximal rise (1.78 mmol/l) was however not different from that in the SC or IM experiments. These results clearly show that plasma glucagon and blood glucose responses are identical after the injection of glucagon whether administered IM or SC. Therefore patients' relatives need not to be trained in intramuscular injection techniques for the administration of glucagon in severe hypoglycaemia.

377. A radioimmunoanalytical and immunocytochemical study on A and B insular cells in response to pinealectomy or pineal denervation

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In an attempt to determine the role of the pineal gland on glucose homeostasis, blood plasma levels of glucose, insulin and glucagon were determined in normal (NR) and diabetic rats (DR; 50 mg/kg streptozotocin intravenously) with pinealectomy, denervation of pineal gland (cervical superior ganglion) and simulated pinealectomy. In addition, the ultrastructure of pancreatic A and B cells was studied. In simulated pinealectomy NR insulin concentrations (200 ± 70 mU/l) were greater than ($p < 0.05$) in pinealectomized (75 ± 25) and gangliectomized (82 ± 50) rats, while glucagon levels (pg/ml) increased ($p < 0.05$) in pinealectomized (268 ± 9) compared with simulated pinealectomy NR (166 ± 53). Significant differences ($p < 0.01$) in plasma glucose concentrations (mmol/l) between DR (15.55 ± 1.94) with pinealectomized DR (17.50 ± 4.27) and gangliectomized DR (20.11 ± 4.44) were observed. Also, plasma glucagon levels of DR (424 ± 159) were smaller than in DR after pinealectomy (845 ± 378, $p < 0.01$) and gangliectomy (1.405 ± 728; $p < 0.05$). In contrast, insulin levels were minimal in all DR-groups. A marked degranulation of the A cells was observed in the islets of pinealectomized rats as determined with immunocytochemical techniques, using 30 K glucagon antiserum. These results suggest a close relationship between pineal gland and the insular cells in both normal and diabetic animals.

378. Parallel changes in glycohaemoglobin levels and in degree of dyschromatopsia in diabetes

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Dyschromatopsia of the tritan type is frequent in diabetic patients free from retinopathy. In a previous cross-sectional study we found a positive correlation between tritanopia and glycohaemoglobin in Type 1 (insulin-dependent) diabetic patients. In the present study, tritanopia and glycohaemoglobin were measured at entry and 3 months later in 29 tritanopic Type 1 diabetic patients (14 males, 15 females), aged 13–35 years (mean 24 years), free from retinopathy at ophthalmoscopy through dilated pupils. Tritanopia was quantified as total score of errors (TS) in the Farnsworth-Munsell 100-Hue test; glycohaemoglobin by chromatographic method. The patients were divided into three groups (A: $n = 9$; B: $n = 9$; C: $n = 11$), according to glycohae-

moglobin levels (mean ± SD): A: from 12.65 ± 3.28 to 7.75 ± 3.14 ($p < 0.0001$); B: from 6.25 ± 2.50 to 12.33 ± 3.11 ($p < 0.0001$); C: from 10.14 ± 4.23 to 11.09 ± 2.26 (NS). The TS in the three groups behaved as follows (mean ± SD): A: from 64.56 ± 49.40 to 46.67 ± 44.58 ($p = 0.01$); B: from 46.68 ± 26.86 to 58.89 ± 21.65 ($0.10 > p > 0.05$); C: from 67.45 ± 49.18 to 69.82 ± 52.77 (NS). These data show that the changes in glycohaemoglobin are closely paralleled by those in TS. We conclude that dyschromatopsia in diabetes can be regarded as a consequence of metabolic derangement, and, as such, it can be reversed, at least in part, by improvement in metabolic control.

379. Effects of glucose intolerance and diabetes on energy expenditure

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The thermic response to glucose is reported to be reduced in patients with diabetes or glucose intolerance. We compared the resting metabolic rate of five obese diabetic women (O-D), five who were glucose intolerant (O-GIT), five who had normal glucose tolerance (O-NGT), and five lean control subjects, and their insulin, glucose and thermic responses after a meal of 75 g glucose. The RMR of O-D was 296 ± 15 mlO_2/min significantly higher than L (200 ± 6, $p < 0.002$). O-GIT (292 ± 9, $p < 0.002$) and O-NGT (254 ± 9, $p < 0.02$) were also increased relative to lean control subjects. The thermic response to glucose was smaller in O-D (7.1 ± 1.8 kcal/150 min) and in O-GIT (6.4 ± 0.8) than in O-NGT (16.7 ± 2) or lean control subjects (14.0 ± 2, $p < 0.01$). During a glucose tolerance test the total energy expenditures of O-D (252 ± 32 kcal/150 min), O-GIT (221 ± 5) and O-NGT (201 ± 9) was significantly higher than lean control subjects (158 ± 4, $p < 0.001$). Thus, although the groups with diabetes and glucose intolerance had a smaller thermic effect after glucose, their higher resting metabolic rate meant that their total energy expenditure was higher than control subjects. The reduced thermic response cannot therefore be an important factor perpetuating the obesity.

380. Biothesiometry – a diagnostic tool for diabetic neuropathy?

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Besides neurophysiological measurements the objective measurement of the vibration threshold (biothesiometry) is used to diagnose diabetic neuropathy. In contrast to neurophysiological measurements biothesiometry is non-invasive and easy to handle. The aim of our investigation was to evaluate how far biothesiometry can replace neurophysiological measurements. Forty Type 1 (insulin-dependent) diabetic patients (mean age 30 ± 13 years, mean duration of diabetes 16 ± 8 years) with external or implanted insulin infusion devices were examined (treatment period range 1–38 months). The carpal, tibial and tarsal vibration thresholds were measured on both sides with a biothesiometer (Krainer, Vienna). At corresponding points of measurement motor and sensory nerve conduction velocity was evaluated (N. medianus, N. suralis, N. tibialis, N. peroneus). Of 110 normal biothesiometry values only 80 motor and 70 sensory neurophysiological values were within the normal range. Of 130 biothesiometry values in the pathological range only 70 motor and 80 sensory neurophysiological values were within the pathological range. Coefficient of variation: biothesiometry 15%, neurophysiological measurements 0.4%. Analysis of our data showed that a diminished sensory vibration threshold is not in accordance with a reduction in motor or sensory nerve conduction velocity. The evaluation of vibration threshold is an unreliable diagnostic tool for diagnosis or quantification of motor or sensory neuropathy.

381. Host immunity in the Bio-Breeding/Worcester rat

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Spontaneous diabetes occurs in 30–60% of Biobreeding/Worcester (BB/W) rats between 60–120 days of age. An autoimmune pathogenesis is suggested by the presence of pancreatic insulitis, prevention by immunosuppression, and adoptive transfer by concanavalin-A (con-A) activated splenocytes from acutely diabetic donors. We analyzed the role of host immunity in BB diabetes in two experiments. Diabetes-resistant (W-line) and Wistar Furth (WF) rats, both $RT1^u$, were lethally irradiated (750 rads) and within 24 h reconstituted with bone marrow (2×10^8 cells) from either diabetes-prone (DP) or W-line donors. Within 6 weeks, spontaneous diabetes occurred in 5/11 W-line and 0/4 WF recipients of DP marrow, but in 0/17 recipients of W-line marrow. Next, 35-day-old DP, or approximately 100 day old Brown Norway (BN, $RT1^n$), Lewis (L, $RT1^l$), WF, and congenic $RT1^u$ Brown Norway (BN.$RT1^u$) were irradiated (750 rads) and reconstituted with DP marrow as before. Three and 7 days later these animals were given 4×10^7 con-A-activated acutely diabetic splenocytes (which theoretically increase effector cells). Within 3 weeks 12/17 DP, 15/18 WF, 0/9 L, 0/9 BN and 3/8 BN.$RT1^u$ rats became diabetic. These data suggest that the bone marrow of DP BB/W rats contains cells necessary for the induction of spontaneous diabetes, and that the induction of diabetes by Con-A-activated spleen cells is associated with MHC restriction.

382. Insulin sensitivity in subjects with insulinoma before and after surgical resection

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Hyperinsulinaemia and altered insulin receptor function may be associated with insulin resistance. Basal hepatic glucose production (HGP), peripheral glucose clearance (employing ^{3}H-3-glucose) and insulin receptor function were measured before and 3 months after successful surgical removal of tumour in four subjects with insulinoma (basal insulin pre-operation: 29 ± 14; post-operation 9 ± 2 mU/l). Paired euglycaemic dose-response curves were developed for each subject. Basal HGP was low pre-operatively (4.3 ± 1.8; versus 14.9 ± 2.8 post-operatively; normal 13.9 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Insulin sensitivity, expressed as right shift of the dose response curve (ED_{50}), was decreased pre- and post-operatively (159 ± 73 versus 148 ± 67; normal 45 ± 2 mU/l). However, insulin responsiveness (V_{max}) remained normal (13.9 ± 2.2 versus 13.8 ± 0.8 post-operatively; normal 16.2 ± 0.9 $ml \cdot kg^{-1} \cdot min^{-1}$). There was no consistent change in monocyte or erythrocyte insulin receptor binding characteristics before or after surgery. These data suggest that chronic hyperinsulinaemia produces a state of insulin insensitivity which appears to be due to a post-receptor defect that is not readily reversed following correction of the hyperinsulinaemia.

383. Quantitation of hepatic extraction of insulin after oral intravenous glucose tolerance tests in normal subjects and Type 2 (non-insulin-dependent) diabetic patients: a non-invasive approach

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To quantitate the uptake of insulin by the liver, peripheral insulin and C-peptide concentrations were measured for 2 h in five normal subjects (N) and in five Type 2 diabetic patients (D) given oral or intravenous glucose (GTT) sufficient to produce superimposable plasma glucose concentrations. Production and post-hepatic delivery rates of insulin were computed by deconvolution analysis of the C-peptide and insulin plasma concentration curves, respectively, using as impulse response functions the plasma disappearance curves of bolus injected C-peptide and ^{125}I-insulin. In normal subjects 24 h basal pancreatic insulin production was 22 U/m^2 (mean of five studies) of which 16 U/m^2 (73% extraction) were cleared by the liver; the same figures in D were respectively 26 and 17 U/m^2 (55%). In the 2-h period after an oral GTT, the pancreas produced, in N, 5.5 U/m^2 of insulin and the hepatic uptake was 3.5 U/m^2 (69%). In D, the production was 5.1 U/m^2 and the hepatic uptake was 3.0 U/m^2 (60%). After an intravenous GTT insulin secretion was significantly lower than after the oral GTT in N (4.2 U/m^2, – 33%, $p < 0.05$) and in D (4.4 U/m^2, – 24%, $p < 0.05$), while hepatic extraction was similar to that observed after the oral GTT (N, 73% versus 69%; D, 64% versus 60%). Fractional hepatic insulin extraction (computed over 10 min intervals) remained fairly constant during the 2-h period of study. These data show that the higher peripheral insulin response to oral compared with intravenous glucose is mainly due to higher insulin production in both normal and diabetic subjects.

384. The domain structure of the adipocyte insulin receptor

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The insulin receptor of adipocytes was labelled by ultraviolet irradiation of reversible complexes with ^{125}I-B2 2-nitro,4-azido-phenylacetyl des-Phe^{B1}-insulin, and the labelled peptides investigated by polyacrylamide gel electrophoresis and autoradiography. Under non-reducing conditions, a 350 kdalton peptide was observed which was converted to a 125 k peptide by thiols. This peptide behaved anomalously on Ferguson analysis, and had a corrected mass of 100 k. Treatment of labelled cells with trypsin (30 mg/l, 30–240 s), or cells with trypsin before labelling, gave reproducible fragmentation patterns highly suggestive of a domain strucutre (350→140 k; 100 k§→95 K, 70 k,

36 k:§ = anomalous). Unlabelled receptors were fragmented more rapidly than labelled, indicating a structural change on insulin binding. No change in binding affinity nor in biological responsiveness to covalently-attached or reversibly-bound insulin was observed after virtually complete fragmentation of adipocyte receptors. Thus only the binding domain seems to be required for signal transmission, and its properties are unaffected by moderate tryptic cleavage. A physical model for the receptor, based on the fragmentation pattern, is presented.

385. Hypoglycaemia and counter-regulation during continuous subcutaneous insulin infusion and during conventional insulin therapy

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To compare the frequency and severity of symptomatic and biochemical hypoglycaemia during continuous subcutaneous insulin infusion (CSII) and conventional insulin therapy (CIT), we treated for 2 months on each regimen, in random order, six Type 1 (insulin-dependent) patients who were not previously troubled by hypoglycaemia on CIT (group A) and five Type 1 diabetic patients who frequently experienced hypoglycaemia (group B). At the end of each insulin regimen, plasma glucose, free insulin and counter-regulatory hormones during insulin-induced hypoglycaemia were measured. In group A, mean ± SD daily plasma glucose was lower on CSII versus CIT (6.3 ± 1.0 versus 8.7 ± 1.7 mmol/l; $p < 0.005$); the frequency of plasma glucose values < 2.5 mmol/l (6.8 versus 7.1%) and of symptomatic hypoglycaemia was not significantly different. In group B glycaemic control also improved on CSII (mean plasma glucose 6.9 ± 1.0 versus 8.7 ± 1.5 mmol/l; $p < 0.02$); the proportion of plasma glucose values < 2.5 mmol/l was not significantly different (5.8 versus 6.3%) but symptomatic hypoglycaemia was reduced by 59% during CSII. There was no significant difference in plasma free insulin, rate of glucose rise or counter-regulatory hormones following insulin-induced hypoglycaemia, but the glycaemic threshold for hypoglycaemic symptoms was lower on CSII in group B (1.3 ± 0.6 versus 1.7 ± 0.5 mmol/l; $p < 0.02$). In conclusion, the frequency of hypoglycaemia on CSII was unchanged in non-hypoglycaemia-prone diabetic patients (group A) but reduced in the hypoglycaemia-prone group (B).

386. Growth hormone stimulates islet cell replication to form expanding monolayers

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It was previously shown that growth hormone (GH) stimulates insulin release and DNA synthesis in newborn rat islets in suspension culture. However, the growth was limited in time, suggesting adaptation to GH, limitation in number of cell divisions or physical limitation in tissue size. To test these hypotheses newborn-rat islets were allowed to attach to a plastic substrate in medium RPMI 1640 supplemented with 2% human serum and exposed to GH for up to 3 months. In the presence of human GH 100–1000 ng/ml an extensive spreading of the islet cells was seen, reaching a monolayer state after 4–6 weeks. The insulin release to the medium increased from about 20 to 200 ng/day per islet and the cell number estimated from the DNA content increased about fivefold after 3 months. At that time the central cell layer rolled up and holes appeared in the islet cell layer. Removal of GH from the cultures likewise resulted in breaking up of each islet colony into smaller colonies. In conclusion, these results indicate that GH promotes attachment, spreading and replication of pancreatic β cells. It is suggested that GH plays an important role in islet formation.

387. Glucose stimulates the accumulation of cadmium in pancreatic β-cells

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In the search for elements suitable for exploring Ca^{2+} movements significant for insulin release, cadmium was studied. Electrothermal atomic absorption spectroscopy was employed for measuring cadmium in β-cell-rich pancreatic islets isolated from obese-hyperglycaemic mice. Endogenous content of cadmium corresponded to 2.5 μmol/kg dry weight. Cd^{2+} (2.5 and 25 μmol/l) evoked a prompt and sustained insulin release. The lower Cd^{2+} concentration did not significantly suppress glucose oxidation. Although not reaching a steady state there was a 60-fold accumulation of Cd^{2+} after 90 min of incubation at Cd^{2+} (2.5 μmol/l). This accumulation was significantly stimulated by both D-glucose and a high K^+. Extracellular Ca^{2+} suppressed Cd^{2+} uptake in a concentration-dependent manner up to 1.28 mmol/l. Only 35% of the cadmium, incorporated after loading with 2.5 μmol/l of the ion, was mobilized during 60 min of incubation in medium containing EGTA (0.5 mmol/l). The efflux was not affected by D-glucose or lowering of the temperature to 4 °C. It is suggested that the glucose-stimulated accumulation of Cd^{2+} in the pancreatic β-cells is similar to that of Ca^{2+} involving activation of voltage-dependent Ca^{2+}-channels.

388. Glibenclamide increases the osmotic resistance of β cells by affecting K^+-mediated volume regulatory mechanisms

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We have investigated mechanisms for sulphonylurea-induced insulin release by studying how glibenclamide interacts with the β-cell plasma membrane. The size of isolated βcells varied with external osmolarity but the swelling induced by hypo-osmolarity was less than expected for perfect osmometric behaviour, i.e. βcells have mechanisms for volume regulation. Glibenclamide (1–200 μmol/l) reduced β-cell swelling in hypo-osmolarity. We tested whether tranpsort of K^+ is involved in volume regulation in the β cells and whether glibenclamide acts on such mechanisms. Reduction of the osmolarity from normal 317 mosm/l to 180 mosm/l reduced the apparent β-cell K^+ content, had no effect on the ouabain-sensitive portion of the influx (Na^+/K^+ pump) but reduced the ouabain-resistant fraction. Hypo-osmolarity also markedly increased the $^{86}Rb^+$ efflux. Glibenclamide (1 μmol/l) in iso-osmolar medium transiently reduced the $^{86}Rb^+$ efflux, at 10 nmol/l to 0.2 mmol/l markedly reduced the ouabain-resistant $^{86}Rb^+$ influx, and at 0.2 mmol/l reduced the apparent K^+ content. Glibenclamide (20 μmol/l) augmented the hypo-osmolarity-induced reduction in apparent K^+ content. These results suggest that the β-cell volume regulation in hypo-osmolarity is in part due to passive K^+ transport and that glibenclamide increases β-cell osmotic resistance by acting on K^+ transport.

389. The muscle of ketotic diabetic man shows a significant production of ketone bodies

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The assumption that ketone body (KB) formation takes place exclusively in liver is based on arteriovenous (A-V) differences of cold acetoacetate (AcAc) and 3-hydroxybutyrate (βOH), a technique which enables only the determination of net balance. In contrast the combined tracer and tracee approach with A-V differences allows the distinction between new synthesis and removal. KB turnover was studied twice in six highly ketonic diabetic patients (D) and in eight normal subjects (N). (1) Forearm KB production (R_p), utilization (R_u), interconversion of AcAc to βOH (R_{21}) and of βOH to AcAc (R_{12}) in D and in N were investigated measuring A-V differences of cold and ^{14}C-AcAc, ^{14}C-βOH with blood flow during continuous infusion of ^{14}C-βOH (2.8 μCi/min) and indocyanine green dye (0.5 mg/min). R_p was 10.5 ± 1.5 μmol/min in D and 0.70 ± 0.49 in N ($p < 0.01$); R_u was 9.1 ± 3.2 μmol/min in D and 1.49 ± 0.37 in N ($p < 0.05$); R_{12} was 3.9 ± 2.2 μmol/min in D and 1.9 ± 0.7 in N, R_{21} was 8.4 ± 1.7 μmol/min in D and 0.8 ± 0.4 in N ($p < 0.01$). (2) Total KB turnover was measured on a second occasion in C and in N injecting ^{14}C-βOH and ^{14}C-AcAc using noncompartmental analysis. In D R_p of AcAc was 455 ± 171 μmol/min and 198 ± 52 in N; R_p of βOH was 1839 ± 318 in D and 241 ± 58 in N. Assuming that muscle is about 64% of the volume forearm and muscle 40% of body weight, it is possible to extrapolate that overall muscle tissue synthesizes about 25% of KB in D and only 2% in N. Secondly the equilibrium between the two KB is shifted towards βOH formation rather than utilization in the forearm of ketotic D.

390. Vitamin D metabolites and vitamin D-binding protein in streptozotocin-induced and spontaneous diabetes of rat

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The interrelationship between vitamin D and insulin secretion was studied. Deficiency of vitamin D results in hyposecretion of insulin and glucose intolerance in rabbits. Conversely, an effect of insulin deficiency on the vitamin D endocrine system may exist in view of the high frequency of osteopaenia in diabetes mellitus. We therefore measured serum 25-hydroxyvitamin D ($250HD_3$), 1,25-dihydroxyvitamin D [$1,25(OH)_2D_3$] and vitamin D-binding protein (DBP) in streptozotocin (STZ)-diabetic Wistar and spontaneously diabetic BB Wistar

rats. $1,25(OH)_2D_3$ was lower in STZ-diabetic rats (♂: 182 ± 38, ♀: 170 ± 33 pmol/l, mean ± SD) than in control rats (♂: 289 ± 58, ♀: 231 ± 40 pmol/l, $p < 0.001$). DBP was also decreased in diabetic rats (♂: 4.9 ± 1.2, ♀: 4.0 ± 0.3 μmol/l) compared with controls (♂: 7.3 ± 1.0, ♀: 5.1 ± 0.4 μmol/l, $p < 0.001$). The diabetic BB Wistar rats showed a 50% reduction in serum DBP (♂: 5.6 ± 1.9, ♀: 4.8 ± 1.1 μmol/l) compared with normoglycaemic animals (♂: 11.2 ± 1.4, ♀: 8.7 ± 1.1 μmol/l, $p < 0.002$). Male diabetic BB Wistar rats had a significantly lower $1,25(OH)_2D_3$ concentration (182 ± 31 pmol/l) than normal rats (267 ± 70 pmol/l, $p < 0.01$) but female diabetic and non-diabetic animals had similar values (151 ± 62 versus 143 ± 38 pmol/l, NS). The concentration of $25OHD_3$ was not altered by diabetes. Circulating vitamin D hormone is thus decreased in STZ-induced and spontaneous diabetes of the rat in association with lowered DBP levels. A sex difference was noted. These findings may have an utmost importance in the pathogenesis of bone disease in diabetes mellitus.

391. Risk of diabetes in Australian Aborigines from the desert

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Previous studies have shown that Aborigines from various coastal regions of Australia are prone to Type 2 (non-insulin-dependent) diabetes when they urbanise. Aborigines are not a homogeneous population and it is not known whether all groups are equally vulnerable to diabetes. To investigate this question, we recently conducted metabolic tests in a cross-section of the adult males from a desert community in north-west central Australia. These people no longer live as hunter-gatherers, but lead a sedentary lifestyle and eat 'western' foods. Sixty-three men, aged 15–68 years, underwent a 75 g oral glucose tolerance test. Although diabetes was not widespread (4 cases), a further 17 men had impaired glucose tolerance (2 h glucose ⩾ 7.8 mmol/l), 30 had fasting hypertriglyceridaemia (⩾ 2 mmol/l), and 40 had hyperinsulinaemia (Δarea under the curve 0–3 h ⩾ 130 $mU \cdot l^{-1} \cdot h^{-1}$). Although the group was not obese (body mass index: 22.7 ± 0.5 kg/m^{-2}), there was a tendency towards increased body mass index with age. Impaired glucose tolerance and hypertriglyceridaemia were both strongly age-related, while hyperinsulinaemia occurred with similar frequency across the age range. These results suggest that as urbanisation proceeds and adiposity increases, these desert Aborigines may be at even greater risk of diabetes than the coastal Aborigines.

392. A comparative study of insulin secretion from pancreatic islets maintained in free-floating and monolayer cultures

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Recently, a tissue culture technique in which pancreatic islets are maintained in culture for varying periods is being used for transplantation. Cultured islets should have a high clinical potential for supplying insulin, glucagon and somatostatin in response to normal physiological demands. This report describes a comparative examination of adult rat pancreatic islets in either monolayer or free-floating cultures. Insulin secretion of freshly collagenase-isolated rat islets and islets which had been maintained in either free-floating or monolayer cultures for 2 weeks was measured during incubation in a medium containing D-glucose (5.5 mmol/l), then in D-glucose (16.7 mmol/l) concentration, and finally in a combination of D-glucose (16.7 mmol/l) and 3-isobutyl-1-methylxanthine (IBMX, 1.0 mmol/l). The insulin secretion of the monolayer islets culture was markly enhanced by the stimulation of glucose at the high concentration and in combination with IBMX. In addition, the monolayer islets had the highest insulin content. These data suggest that the most favourable conditions for long-term survival of isolated islets in culture may be obtained when the islets form a monolayer.

393. Paratopic segmental pancreas transplantation in patients with diabetic nephropathy

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We report four patients with diabetic nephropathy who received simultaneous segemental pancreas and renal transplants. The pancreas grafts were transplanted by a novel technique in which exocrine secretion drains into the body of the stomach and endocrine secretion into the splenic vein. The pancreas grafts were positioned close to the patients own pancreas, in a paratopic position. Immunosuppression was with Cyclosporin A and Campath 1, a monoclonal antibody active against mature T and B lymphocytes. Insulin was discontinued within 24 h of surgery in all four patients. Intravenous and oral glucose tolerance tests (GTT) and 24-h metabolic profiles were performed between days 7 and 30 post-operatively. All patients had abnormal intravenous GTT K values, and one had a normal oral GTT; however the 24-h blood glucose profiles were virtually normal. Insulin, C-peptide and proinsulin data will be presented. The patients are insulin independent at 3, 2½ and 2 months after receiving paratopic pancreas allografts, although all patients had episodes of renal graft rejection which required corticosteroid treatment. This technique allows venous drainage of the pancreatic graft into the portal system and may result in improved metabolic control compared with previous methods of pancreas transplantation.

394. Endothelial cell membrane is a glucocorticoid regulated barrier for glucose

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The interactions of elevated blood glucose and endothelial cells are poorly understood. To elucidate pathophysiological aspects, we investigated some fundamentals of endothelial glucose metabolism using primary cultures of porcine aortic endothelial cells as a model. The effects of glucose concentrations and hormones on glucose consumption, lactate, pyruvate, sorbitol and fructoseformation were studied leading to the following results: endothelial cells have a high glycolytic activity which is saturated far below physiological blood glucose levels ($K_m < 1$ mmol/l). Nearly all glucose taken from the medium was recovered as lactate and pyruvate. Glucocorticoids reduced glucose catabolism as a function of their concentration. Insulin, adrenaline, and glucagon did not influence glucose consumption. Studies with the non-metabolizable analogue, 3-0-methyl-glucose, revealed that glucocorticoids slowed down glucose uptake into the cell. Hence, the passage of glucose through the endothelial cell membrane was the rate-limiting step of glucose utilization. Consequently, endothelial cells do not accumulate sorbitol under hyperglycaemic conditions, although they contain aldose reductase activity. Artificial sorbitol accumulation and fructose formation can be induced by high lactic acid concentrations in the incubation medium which cause inhibition of glycolysis. Sorbitol pathway then acts as an overflow mechanism for free intracellular glucose.

395. A Golgi-derived clathrin-coated membrane compartment is associated with the proteolytic maturation of proinsulin

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When the intracellular transit, or the susceptibility to proteolytic cleavage, of radiolabelled proinsulin polypeptides is perturbed by monensin or amino-acid analogues, respectively, gel chromatography shows an inhibition of the conversion of radiolabelled proinsulin to insulin and high resolution autoradiography reveals that the intracellular radioactive material is associated with a coated membrane compartment related to the Golgi apparatus. This compartment, in the pancreatic β cell studied, comprises both coated Golgi cisternae with condensing granule cores and individual coated secretory granules; the clathrin-like nature of the coat was assessed with an anti-clathrin antiserum detected by the protein A-gold method. Under both monensin or analogue treatment the non-coated (storage) secretory granules do not become significantly labelled. These data suggest that the effective conversion of proinsulin to insulin is linked to an unperturbed passage through a Golgi-related coated compartment and its subsequent maturation into non-coated secretory granules. A comparable role of clathrin-coated membrane compartments of the Golgi in other polypeptide-secreting cells where the definitive secretory product results from the proteolytic cleavage of a precursor seems likely.

396. Opposite effects by glucose on binding to muscarinic receptors on pancreatic A and B cells

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Glucose inhibits glucagon (A cell) and stimulates insulin (B cell) secretion evoked by other secretagogues, e. g. acetylcholine. We investigated whether effects on secretion are paralleled by opposing effects of glucose on muscarinic receptors. ^{3}H-methylscopolamine was used to detect and compare binding to cultured islets of streptozotocin-treated (A-cell-rich islets) and untreated (B-cell-rich islets) from guinea pigs. After 3 days of culture in 3.3, 5.5 or 11 mmol/l glucose, binding was assessed under the same conditions used in secretion studies.

Specific binding was significant after 1 min and reached a plateau after 10 min for both types of islets. Specific binding was higher in A-cell-rich islets cultured at 5.5 mmol/l glucose (1.81 ± 0.21 dpm/nl islet volume) compared with islets cultured at 11 mmol/l glucose (0.51 ± 0.11 dpm/nl; $p < 0.01$). In contrast, binding was increased by glucose in normal islets: 0.59 ± 0.10 dpm/nl after 5.5 mmol/l glucose and 1.30 ± 0.20 dpm/nl after 11 mmol/l glucose ($p < 0.01$). Glucagon release by acetylcholine (10 μmol/l) was more stimulated from A-cell-rich islets when cultured at 3.3 mmol/l glucose (4.0 ± 0.5 to 15.4 ± 1.6 pg/islet per h) than when cultured at 11 mmol/l glucose (6.3 ± 0.7 to 12.2 ± 1.9 pg/islet per h; $p < 0.05$). Thus, (1) both A and B cells appear to contain muscarinic receptors, (2) long-term glucose environment exerts opposite effects on A- and B-cell binding, and (3) inhibition of A-cell binding is correlated with reduction of acetylcholine-induced glucagon release.

397. Quantitative studies of glomerular ultrastructure in juvenile diabetic patients with incipient nephropathy

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Glomerular structural quantities were determined in a series of nine juvenile diabetic patients with incipient nephropathy (IN) defined as urinary albumin excretion rate between 30 and 600 ug/min, glomerular filtration rate (GFR) above 100 ml/min per 1.73 m^2, and a normal blood pressure. The data were compared with those obtained in diabetic patients with a duration of ⩾ 5 years (D5) and in diabetic subjects with nephropathy (NP), i. e. albuminuria and decreased GFR. In the present series the mean duration of diabetes was 13 years (range 9–21 years), and the mean age was 23 years (range 16–35 years). Glomerular basement membrane thickness was 527 nm (coefficient of variation (CV) 0.15), a value in between the D5 and NP-groups. Over the spectrum of duration of diabetes (3–29 years) in all three groups, there was a statistically highly significant correlation between duration and basement membrane thickness ($r = 0.71$, $n = 27$)-contrary to what has recently been reported. The width of epithelial foot processes in the IN-group was 334 nm (CV 0.11) and thereby clearly increased compared with the normal value (224 nm, CV 0.06). The mesangial enlargement is as yet only determined as the fractional volume within the glomerular tuft. The results in the groups of D5, IN and NP were (mean and (CV)): 0.34 (0.09), 0.41 (0.12), and 0.57 (0.17). The fraction of capillary surface facing peripheral basement membrane was 0.67 in normal subjects, and 0.65 and 0.47 in IN and NP, respectively.

398. Electrical activity of islets of Langerhans evaluated with tetraphenylphosphonium: relationship to calcium fluxes and insuiln secretion

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The electrical activity of the islets of Langerhans was investigated using an organic lipid soluble cation: tetra-phenylphosphonium (TPP^+). In each experiment, approximately 30 pre-incubated rat islets, isolated by collagenase, were transferred to a glucose-free medium containing TPP^+ (50 μmol/l). Islet uptake of TPP^+ (3.28 ± 0.07 μmol/mg islet protein, $n = 28$) was immediate; and then rapidly stabilized. In this stable period, addition of glucose (20 mmol/l) produced: (1) immediate release of TPP^+ (0.73 ± 0.02 μmol/mg protein, $n = 8$); (2) increased calcium uptake by islets; (3) increased insulin secretion (10.7 ± 0.84 ng/mg protein per min, $n = 10$ versus 2.03 ± 0.14 ng/mg protein per min, $n = 12$ in control experiments). Arginine (20 mmol/l) induced: (1) immediate TPP^+ release, but smaller than for glucose (0.32 ± 0.01 μmol/mg protein, $n = 10$, $p < 0.001$); (2) transient release followed by re-uptake of calcium; (3) less insulin secretion than glucose (7.0 ± 0.24 ng/mg protein, $n = 10$, $p < 0.01$). Fructose (10 mmol/l) had no effect on TPP^+, flux of calcium, or on insulin secretion (2.24 ± 0.29 ng/mg protein per min, $n = 6$). Release of TPP^+ and insulin secretion were highly correlated ($r = 0.902$, $n = 28$, $p < 0.001$). In all experiments the viability of islets was assessed by O_2 consumption. In conclusion, study of the electrical activity of islets of Langerhans TPP^+ is a useful tool. Changes in their electrical activity seem to be related to calcium uptake and insulin secretion.

399. Glucose intolerance as a determinant of excess mortality in diabetic patients following acute myocardial infarction

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We have tested the hypothesis that abnormal glucose homeostasis is a prime determinant of excess mortality in diabetic patients following acute myocardial infarction (AMI), and that the prior mode of treatment confers no extra risk. On admission HbA_{1c} was determined by iso-electric focussing in 301 patients with documented AMI. The interassay co-efficient of variation is 5% with 95% confidence limits of 4.07–7.79%. 34 patients had previously diagnosed diabetes mellitus. 28 had an $HbA_{1c} \geqslant 7.8\%$, of whom 11 died (mortality 39%). There was no significant difference in the distribution of diet, oral or insulin therapy between survivors and deceased ($\chi^2 = 1.48$, $p > 0.1$). 32 patients not known to have diabetes had an $HbA_{1c} \geqslant 7.8\%$, and 13 died, a mortality (41%) significantly greater ($p < 0.05$) than in the remaining 235 patients (mortality 24%). There was no significant difference between age and relevant previous medical history between groups. The similar mortality in treated and untreated groups whose $HbA_{1c} \geqslant 7.8\%$ suggests that glucose intolerance per se is a prime risk factor for excess mortality following AMI in diabetic patients, and that prior treatment with hypoglycaemic agents is unlikely to add any additional risk.

400. Pharmacokinetics of human, porcine and bovine Ultralente insulins

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Six normal subjects received subcutaneous human (HI), porcine (PI) and bovine (BI) Ultralente insulin (0.30 U/kg) and diluent (control) in random order. Plasma glucose, C-peptide and insulin (IRI) were measured 32 h post-injection. Incremental insulin levels due to exogenous insulin were calculated using the formula:
$IRI_{exogenous} = IRI_{observed} - IRI_{endogenous}$; $IRI_{end} = \text{C-peptide}_{obs} \times R$ (R = control day, IRI: C-peptide). After insulin injection little change occurred for 6 h. From 10 h onwards glucose was significantly lower ($p < 0.05$–0.001) with HI compared to BI insulin. PI insulin produced an intermediate response up to 16 h with subsequent values similar to those observed with BI insulin from 24–32 h. Incremental insulin values were higher ($p < 0.05$–0.01) following HI compared to BI insulin between 2–22 h after injection. Intermediate values were observed with PI insulin for 24 h, becoming identical to BI insulin from 28–32 h. Peak mean insulin values for HI, PI and BI were 0.053, 0.044 and 0.023 nmol/l at 14, 16 and 18 h respectively reaching 0.022, 0.013 and 0.012 nmol/l respectively at 32 h. Consideration must be given to the differences between HI and BI in clinical practice.

401. Comparison of the hypotensive and metabolic effects of metoprolol and a high fibre/low sodium/low fat diet in diabetic hypertensive subjects

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Beta blocker therapy is standard treatment in essential hypertension. However, adverse effects on lipid and carbohydrate metabolism have been observed. We have reported previously that a diet of high fibre, low sodium and low fat is hypotensive in hypertensive diabetic patients. We have therefore compared changes in blood pressure, glycaemic control and lipid levels over 3 months in matched hypertensive diabetic patients receiving metoprolol (age 51.7 ± 6.2 years; ideal body weight 126.3 ± 14.7%; 13 males, 8 females) or this modified diet (age 53.1 ± 5.3 years; ideal body weight 133.7 ± 20.0%; 12 males, 9 females). Both groups had a decrease in systolic (metoprolol: 11.2 mmHg, $p < 0.02$; diet: 19.6 mmHg, $p < 0.001$) and diastolic pressure (metoprolol: 11.5 mmHg, $p < 0.001$; diet: 11.5 mmHg, $p < 0.001$). In addition, dietary therapy resulted in decreased glycosylated haemoglobin (2.1%, $p < 0.01$) and weight (3.1 kg, $p < 0.001$). On metoprolol no significant changes were observed. In hyperlipidaemic patients (cholesterol > 7.1 mol/l or triglyceride > 2.1 mol/l) dietary manipulation decreased triglyceride ($p < 0.02$) and glycosylated haemoglobin ($p < 0.01$). No significant changes occurred on metoprolol. We conclude that this modified diet has a similar hypotensive effect to metoprolol with additional improvement in cardiovascular risk. These data suggest this diet may be preferred initial treatment for hypertensive diabetic patients.

402. Computer analysis of heart rate variability in the preclinical detection of diabetic autonomic neuropathy

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Alterations in sympatho-vagal control of the heart may be an early indicator of autonomic neuropathy. Computer analysis of heart rate variability provides a quantitative assessment of cardiac neuropathy, through the calculation of the power spectral density (PSD) of the variability of RR intervals on continuous ECG recording. In 15 normal subjects (aged 35 ± 4 years, heart rate 69 ± 2 beats/min, systolic blood pressure 117 ± 3 mmHg), the RR variance was high (2.5 ± 0.6 × 10^{-3} s^2) and the PSD showed three major peaks at frequencies: P_1 = 0.07, P_2 = 0.12, P_3 = 0.23 cycles/beat) P_2 and P_3 were associated with vagal and P_1 with sympathetic activity. P_1 accounted for only 30 ± 4% of total variability. However with a non-hypotensive orthostatic stimulus (tilting), P_1 became predominant, increasing by 286 ± 57%. In ten diabetic patients without complications in good metabolic control (similar age and haemodynamics), RR variability was reduced (1.1 ± 0.4 × 10^{-3} s^2) and PSD was altered, as P_1 was greater at rest (49 ± 9% of total variability) and increased only 66 ± 25% with tilting. Thus, computer analysis of RR variability appears a sensitive tool for quantifying the early changes in sympatho-vagal control of the heart in the course of diabetes without complications.

403. Pharmacological characterization of K channels in pancreatic β-cells

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K-channels of βcells are the target for clinically useful stimulators (tolbutamide) or inhibitors (diazoxide) of insulin release. To characterize them better, we studied the effect of potent inhibitors of K-permeability in nerve cells on ^{86}Rb efflux and insulin release from mouse islets. Tetraethylammonium and quinine decreased ^{86}Rb efflux and augmented insulin release, but 4-aminopyridine, 3,4-diaminopyridine and apamine were ineffective. Caesium ions produced complex effects. Addition of Cs (4.8 mmol/l) to a K-medium decreased ^{86}Rb efflux and increased insulin release. Both changes were progressively reversible and only the effect on release was glucose-dependent. Substitution of Cs for K rapidly decreased ^{86}Rb efflux; in the presence of glucose, however, the effect was soon masked by a secondary increase. At glucose ≥ 7 mmol/l, insulin release was potentiated in a Ca-dependent manner. Return to a K-medium caused a marked and transient fall in ^{86}Rb efflux and insulin release that was prevented by ouabain. The effects of Cs can be explained by a decrease in K-permeability and a blockade of the Na-pump if K is omitted. Analogies and differences between K-channels in β and nerve cells may help to identify the channels sensitive to insulinotropic agents and to elucidate the mechanism whereby these latter affect K-permeability.

404. Impaired autoregulation of glomerular filtration rate in Type 1 (insulin-dependent) diabetic patients with nephropathy

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The effect of acute arterial blood pressure lowering upon kidney function in diabetic nephropathy was studied in 13 long-term Type 1 (insulin-dependent) diabetic patients. Ten healthy subjects and five short-term Type 1 diabetic patients without nephropathy served as controls. Renal function was assessed by the glomerular filtration rate (GFR, single bolus ^{51}Cr-EDTA technique) and urinary albumin excretion rate (radial immunodiffusion). The study was performed twice within 2 weeks with the subjects receiving an intravenous injection of either clonidine (225 μg) or saline. The arterial blood pressure was nearly the same in the diabetics with nephropathy (mean 136/88 ± 11/5 mmHg, and in the non-diabetic control subject (mean 140/92 ± 25/15 mmHg). The clonidine injection induced the same reduction in mean arterial blood pressure in all three groups (16–18 mmHg). While GFR and urinary albumin excretion rate remained unchanged in both control groups after clonidine injection, GFR diminished from 78 to 71/ml/min per 1.73 m^2 ($p < 0.01$), and urinary albumin excretion declined from 1707 to 938 μg/min ($p < 0.01$) in the patients with diabetic nephropathy. Our results suggest that intrinsic vascular (arteriolar) mechanisms underlying the normal autoregulation of GFR, i.e. the relative constancy of GFR that occurs in response to rather wide variations in perfusion pressure is defective in diabetic nephropathy.

405. Influence of physiological increments in plasma calcitonin concentration upon β-cell function in normal man

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Previous studies have suggested a diabetogenic action of pharmacological doses of calcitonin in man. The aim of the present study was to evaluate the influence of physiological increments in plasma calcitonin concentrations upon β-cell function in normal man. Ten normal subjects received an oral glucose tolerance test (100 g) under basal conditions and during an infusion of human calcitonin at a dose of 0.08 μg/min. An additional eight normal subjects received an arginine test (30 g infused over 30 min) with or without the same calcitonin infusion. The dose of calcitonin used raised the plasma calcitonin concentration to a mean value of 150 ± 37 pg/ml, levels which are found in response to a meal. Calcitonin infusion significantly decreased ($p < 0.01$) the insulin response to oral glucose and produced a deterioration in glucose tolerance. Similarly, insulin responses to arginine were significantly ($p < 0.05$) reduced by calcitonin. Even the acute insulin response (2–10 min) to intravenous glucose (20 g) challenge was significantly ($p < 0.01$) reduced by this calcitonin dose. These data to indicate that calcitonin plays a role in the negative modulation of β-cell function under physiological conditions. Thus, the ingestion of nutrients stimulate gastrointestinal hormone and calcitonin secretion, which have opposite effects on insulin secretion.

406. Characterisation of secondary failure of glibenclamide therapy

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The mechanisms of secondary failure of sulphonylurea therapy are unclear. We assessed 24 patients who failed on glibenclamide therapy (glycosylated haemoglobin (GHb) > 10%) despite glibenclamide 30 mg/day following at least 6 months of adequate control. Endogenous insulin secretion was estimated following stimulation with glucagon (1 mg) intravenously. Two groups were defined – group A (six males, two females, mean ± SD age 55.4 ± 13.2 years) with stimulated C-peptide < 0.3 nmol/l; group B (three males, 13 females, mean age 61.9 ± 7.6 years) with stimulated C-peptide > 0.3 nmol/l. Age and GHb did not differ significantly; the sex difference was highly significant ($p = 0.006$). Duration of diabetes was significantly less in group A (2.8 ± 2.4 years) than in group B (7.1 ± 6.7 years; $p < 0.02$, Wilcoxon). Patients in group B were more obese (body mass index (weight ÷ height^2 27.2 ± 3.4 kg/m^2) than those in group A (22.5 ± 3.0 kg ÷ m^2; $p < 0.01$) and required a larger dose of injected insulin to achieve control (group B 46.9 ± 13.2 U/day, group A 29.7 ± 12.0 U/day, $p < 0.02$). Secondary failure of glibenclamide therapy thus has two mechanisms – some patients, predominantly male and thin, have reduced insulin secretion while others, mostly female and obese, have substantial endogenous insulin secretion and are presumably insulin resistant. The shorter duration of diabetes in the insulin-deficient group suggests that the two groups are not simply different stages of evolution of the disease but represent two different disease patterns.

407. The major proglucagon fragment is composed of two glucagon-like sequences and represents a new secretory product of pancreatic islets

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From biosynthetic studies on proglucagon formation and conversion in pancreatic islets, a conversion product of 10 kDal was identified. By immunoprecipitation and peptide mapping, this major proglucagon fragment (MPGF) was recognized as a glucagon-free portion of the prohormone. Moreover, by pulse-chase experiments and by its high abundance in pancreatic islets, MPGF was identified as an end-product of proteolytic prohormonal processing. This peptide was isolated from large batches of isolated rat islets and its amino-acid composition was determined. With respect to the primary structure of mammalian proglucagon, as deduced in other laboratories from the preproglucagon c-DNA sequence, MPGF was recognized as the C-terminal half of the prohormone comprising 2 sequences which are structurally homologous to glucagon. Thus, these two glucagon-like sequences are not proteolytically separated in pancreatic A cells but form, together with a short spacer sequence in between, a new islet protein. Evidence for the secretion of MPGF along with glucagon is presented.

408. Comparison of human and porcine NPH insulins in the treatment of insulin-dependent diabetes mellitus

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It has previously been shown that absorption rates of porcine (Insulatard) and human (Protaphane HM) NPH-insulins are comparable. The aim of the study was to test these finding in a double-blind cross-

over clinical study. Twenty-two patients gave informed consent. After a run-in period in which the patients were instructed in home blood glucose monitoring, the patients were randomized to either group A (Insulatard + Actrapid MC) or group B (Protaphane HM + Actrapid HM). After 8 weeks of treatment (phase 1) the insulin regimens were reversed in the two groups (phase 2). Mean fasting blood glucose in group A was 8.82 versus 7.71 mmol/l ($p < 0.05$ phase 1 versus phase 2), in group B 8.93 versus 8.57 mmol/l (NS). No significant differences were found at the other blood glucose measuring time points, but after breakfast there was a tendency to a better glycaemic control on Protaphane HM + Actrapid HM compared with Insulatard + Actrapid MC. No significant differences were found between groups A and B. The insulin dose remained constant, and no adverse reactions were reported with either insulin. In conclusion, porcine and human NPH-insulins have been found comparable in the treatment of diabetes in clinical practice.

409. Characterization of mechanisms responsible for the insulin resistance of patients with uraemia: in vivo and in vitro experiments

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The basis of insulin resistance in uraemia is not clarified. In the present study we examined: (1) in vivo insulin dose-responses in uraemia; (2) adipocyte insulin receptors and the effect of insulin on intracellular glucose processing in chronic renal failure. A three-step euglycaemic clamp was done in 14 uraemic and 10 healthy subjects. Three rates of insulin were infused each during 120 min: 0.5, 2.0 and 4.0 $mU \cdot kg \cdot^{-1} min^{-1}$. The last 30 min glucose disposal rates were consistently lower in uraemic than in control subjects (2.3 ± 0.3 versus 6.6 ± 0.8; 7.8 ± 0.6 versus 13.2 ± 1.1 and 9.6 ± 0.7 versus 15.5 ± 1.0 $mg \cdot kg \cdot^{-1} \cdot min^{-1}$; all $p < 0.001$). Insulin levels were similar in the two groups, but those required to elicit half-maximal response were higher in uraemic subjects (83 ± 5 versus 54 ± 8 mU/l, $p < 0.01$). ^{125}I-insulin binding, non-insulin and maximal insulin stimulated ^{14}C-D-glucose transport and conversion of ^{14}C-D-glucose to total lipids were identical in eight uraemic and matched control subjects. However, in uraemic patients insulin concentrations causing half-maximal glucose transport and lipogenesis were shifted threefold to the right ($p < 0.02$), indicating a decreased sensitivity to insulin. In conclusion, uraemic insulin resistance is the result of severely depressed insulin action localized at post-binding cellular sites.

410. Pancreatic islet cells produce leukotrienes

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Lipoxygenases catalyze the conversion of arachidonate to hydroxy- and hydroperoxy-eicosatetraenoates (HETE, HPETE) and leukotrienes (LT). We have shown that the administration of inhibitors of lipoxygenases in vitro inhibit, and LT stimulate insulin release. We searched for evidence for LT biosynthesis in islet cells. Freshly isolated rat islets or sub-clones of insulin-secreting rat islet tumour cells in continuous culture (RIN-5F) were loaded with ^{3}H-arachidonate, then washed and incubated in RPMI/Hepes media for 1 h at 37 °C in 5% CO_2 and air. Media were extracted and submitted to high-pressure liquid chromatography. The 5-µm C-18 reversed phase column was eluted with acetonitrile : water : trifluoroacetic acid, isocratic 31 : 69 : 0.1 for 40 min, followed by a linear gradient increase of acetonitrile starting at 45 : 55 : 0.1 over 2 h. The proportions of radioactivity were: with islets 59%, LTB_4 plus LTC_4 and 2% 12-HETE, and with RIN-5F 29% LTB_4 plus LTC_4 and 44% 5-HETE. In conclusion, (1) islet cells are capable of producing leukotrienes; (2) leukotrienes in islets are likely to function as amplifiers of insulin secretion.

411. Insulin action during fasting and refeeding in rats: assessment by the glucose clamp technique

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Many lines of evidence are in favour of a major role of insulin in the physiological adaptation to fasting and refeeding, but the alterations of biological effects of insulin in vivo are not well known. Euglycaemic hyperinsulaemic clamps were used to study the sensitivity of tissues to various insulin levels (0.15, 0.3, 0.5, 5 mU/ml) in rats having free access to food and in 72-h fasted and 72-h refed rats. Glucose production and utilization were measured using (^{3}H) glucose. In the basal state, glucose turnover and insulin levels were 46 ± 3 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ and 0.13 ± 0.01 mU/ml in fed rats; 38 ± 2 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ and 0.03 mU/ml in fasted rats; 59 ± 3 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ and 0.2 ± 0.03 mU/ml in refed rats. At maximal insulin concentration (5 mU/ml), glucose utilization was less increased in fasted rats ($+72$ $\mu mol \cdot min^{-1} \cdot kg^{-1}$), than in the two other groups ($+149$ in fed and $+121$ $\mu mol \cdot min^{-1} \cdot kg^{-1}$ in refed rats). Hepatic glucose production was totally inhibited in fed and refed rats for insulin concentrations of 0.3–0.5 mU/ml, but was decreased only by 50% in fasted rats at insulin concentrations of about 5 mU/ml. These data indicate the presence of a state of insulin resistance during fasting which is partly restored after 72 h of refeeding in rats.

412. Effects of guar on diabetes and lipids – food and pharmacology compared

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Poor palatability has restricted the therapeutic application of guar gum. We have developed a guar bread (GB) almost identical to commercial bread and previously shown its acceptability. Twelve non-insulin dependent diabetic patients completed a randomised cross-over study of three 6-week periods. Their usual bread was replaced by either control bread (0% guar), GB (100 g guar/kg flour), or control + guar granulate (GG) (Meyhall Chemical). Daily guar intake was 6 g from GB and 10 g from GG (5 g twice daily). After GB there were falls in mean fasting plasma glucose (8.7 to 8.1 mmol/l, NS), HbA_{1c} (9.6 to 9.2%, $p < 0.05$; normal < 8.5%), total cholesterol (6.41 to 5.80 mmol/l, $p < 0.001$) and triglycerides (2.08 to 1.73 mmol/l, $p < 0.05$); the HDL/LDL ratio rose by 0.030 ± 0.011 ($p < 0.05$). After GG there were falls in glucose (8.5 to 8.0 mmol/l, NS), HbA_{1c} (9.8 to 9.4%, $p = 0.02$), cholesterol (6.05 to 5.56 mmol/l, $p < 0.02$) and triglycerides (1.97 to 1.62 mmol/l, $p < 0.05$); the HDL/LDL ratio rose by 0.038 ± 0.013 ($p = 0.02$). After control bread there were no significant changes in biochemistry; body weight and nutrient intakes were unchanged throughout. Ten patients preferred GB to GG. A lower than conventional dose of 6 g guar in GB was as effective as 10 g GG. Incorporating guar into food enhances its metabolic benefits and a search for further acceptable diet products is being pursued.

413. Cysteamine rapidly decreases pancreatic islet somatostatin in vitro

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Previous studies indicate that cysteamine (CA; β-mercaptoethylamine) decreases somatostatin in the pancreas of the rat in vivo. CA may therefore be useful for studies of intra-islet regulation of hormone release. In the present study injection of CA (60 mg/kg body weight) into mice decreased somatostatin of isolated islets to < 50% in 4 h ($p < 0.001$). Exposure of isolated mouse islets to CA (100 mg/l) in vitro for either 1 h only, or for 0.5 h followed by 3.5 h incubation in control medium, decreased the islet somatostatin content by 55–90% ($p < 0.001$). There was no change in the islet content of insulin or glucagon. Measurements of insulin release from isolated islets pre-incubated for 1 h with CA 100 mg/l showed increased basal release ($p < 0.001$) and attenuated glucose stimulation. The islet glucose oxidation rate remained normal after 60 min exposure to CA 10 mg/l but was markedly decreased ($p < 0.001$) after CA 100 mg/l. It is concluded that CA rapidly decreases islet somatostatin in vitro. However, possible direct effects of CA also on the islet β cells could make this experimental model less suitable for studies of paracrine influences of somatostatin.

414. Effect of dopaminergic blockade on glucose and arginine-induced insulin release

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Dopamine has been demonstrated to affect the release of insulin in man. Because dopamine exerts its effects through multiple receptor classes and no data are available about the effects of a single dopamine receptor type on insulin release, we evaluated the insulin response during D2 receptor blockade with domperidone in man. Ten subjects were randomly submitted to intravenous glucose (0.33 g/kg) and to arginine infusion (30 g/30 min) with and without the simultaneous infusion of domperidone (0.005 mg/kg). The infusion of domperidone started 30 min before the glucose or arginine loading. The results show an increase in the release of insulin during the infusion of domperidone after intravenous glucose at: 3 min (115.3 ± 9.8

versus 86.0±8.3 mU/l, $p<0.001$); 5 min (96.7±6.4 versus 80.7±5.5 mU/l, $p<0.001$); 8 min (71.6±5.4 versus 61.0 mU/l, $p<0.001$); and 10 min (56.2±5.5 versus 47.5±3.8 mU/l, $p<0.05$). We did not find any variation of insulin levels in the baseline samples, during the late phase of insulin release after glucose and during the arginine load. Our data show that D2 receptor blockade increases the rapid phase of β-cell response to glucose, without affecting the basal release of insulin and the response to arginine. These data are consistent with a specific inhibitory role of D2 dopamine receptors on rapid phase of the β-cell response to glucose.

415. Regulation of receptors for platelet-derived growth factor by insulin-like growth factor I

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The platelet-derived growth factor (PDGF) and the growth hormone dependent insulin-like growth factor I (IGF I) are growth factors for arterial smooth muscle cells. IGF I is elevated in sera of diabetic patients with proliferative-exudative disease. Both factors may be important for the pathogenesis of atherosclerosis. We studied the binding of ^{125}I-PDGF to cultured arterial smooth muscle cells and examined the influence of IGF I on this binding. Binding studies were carried out with cell layers in 0.1 mol/l Hepes buffer (pH 7.4) with 0.2% human serum albumin. The effect of IGF I on ^{125}I-PDGF binding was studied after a pre-incubation of the cells with IGF I for 1–5 h. Specific binding of ^{125}I-PDGF was 10% and became irreversible at 37 °C. Half-maximal displacement by PDGF at 4 °C occurred at 6 nmol/l. Pre-incubation of the cells with IGF I caused an increase in ^{125}I-PDGF binding. A Scatchard analysis indicated an increase in the number of binding sites for ^{125}I-PDGF. These results suggest that both factors are involved in the regulation of growth by their interaction on the cell surface itself.

416. Effects of arginine on pancreatic hormone secretion and glucose kinetics in freely-moving rats

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The secretion and effects of pancreatic hormones were assessed in freely-moving rats fitted with a permanent portal catheter. Arginine was infused intravenously for 20 min after a 4 h fast. Glucose, insulin (IRI) and glucagon (IRG) were assayed in portal and peripheral plasma. Glucose kinetics were measured using (3-^{3}H)-glucose. In the basal state, concentrations in portal and peripheral plasma were respectively: glucose 7.92±0.16 versus 7.42±0.11 mmol/l (NS); IRI 156±15 versus 126±8 mU/l (NS); IRG 894±135 versus 400±32 pg/ml ($n=17$, $p<0.001$). Glucose turnover was 10.3±0.7 mg·kg^{-1}·min^{-1}. Blood glucose did not change during arginine infusion, but decreased after cessation of infusion. This resulted from a similar, rapid rise in glucose production and utilization (+33%) during arginine infusion, followed by a sustained increase in glucose utilization only. Concomitantly, IRI and IRG concentrations displayed a biphasic increase. No porto-peripheral gradient was observed for IRI, while a clear-cut gradient persisted for IRG during the first 10 min of arginine infusion. Similar glucose kinetics and hormone changes in peripheral plasma were observed in control rats, without a portal catheter: portal vein catheterization does not impair islet or liver function. Comparison with previous results obtained after a 16 h fast suggests that pancreatic hormone uptake by the liver is strongly influenced by the nutritional state.

417. Diabetes mellitus and asymptomatic bacteriuria

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The incidence of asymptomatic bacteriuria was studied in 270 patients (127 males and 143 females, mean age 62 years) with diabetes mellitus and compared with 118 healthy control subjects matched for age and sex. In 83 patients (30%) bacteriuria-aggravating factors were encountered as follows: renal colic in 30, bladder catheterisation in 35, prostate hypertrophy in 11 and prostatectomy in 7. The total incidence of bacteriuria ($\geq 10^5$ cfu/ml) was 17% but only 5.9% in controls subject ($p<0.01$ after applying the Mantel-Haenszel method) with stratification by sex and age. However, bacteriuria was identified only in 12 (15%) out of the 83 patients with predisposing factors. The screening for antibody-coated bacteria in urine, in order to localize the site of bacteriuria, was strongly positive in 71% of the diabetic patients. In conclusion, (1) the incidence of asymptomatic bacteriuria in diabetes is much higher than that expected in the non-diabetic population; (2) bacteriuria is localized mainly to the upper urinary system; (3) duration of diabetes, levels of glycaemia and insulin dependence do not influence the incidence of bacteriuria and (4) routine screening for detection of bacteriuria with urine cultures seems to be justified during the periodic assessment of diabetic control.

418. Use of purified βcells and cyclosporin-A in allografting diabetic rats

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Rejection of islet allografts has been attributed to the immunogenicity of Ia-presenting non-endocrine cells lodged in donor tissue. Purified pancreatic βcells might thus lead to successful transplantation across an MHC barrier provided their graft is capable of reversing the diabetic state. Single βcells were purified from R/A rat pancreas and aggregated into clumps of diameter 50–500 μm. After a 4 day culture, β-cell aggregates were injected intraportally in streptozotocin-diabetic R/A rats. Fasting plasma glucose levels normalised within 1 week, and multiple insulin-containing aggregates were identified in liver sections. Allografts of R/A islet tissue were performed in streptozotocin-diabetic BN-rats. Transplantation of cultured intact R/A islets immediately reversed the diabetic state in BN-recipients, but all rats became hyperglycaemic again within 3 weeks. Injection of purified R/A β-cell aggregates induced normal fasting glycaemia for periods varying from 2 to 15 weeks. A 15-week-long normoglycaemia was installed after allografting islets or β-cell aggregates in BN-recipients that were treated for 5 weeks with cyclosporin-A. In conclusion, purified β-cell aggregates can reverse a diabetic state. As a group, β-cell allografts are better tolerated than islet allografts. Tolerance to islet and to pure β-cell allografts is also induced by cyclosporin-A and is maintained after omission of the immunosuppressive agent.

419. Suppression of growth hormone and somatomedin C secretion by long-acting somatostatin analogue (SMS 201-995) in Type 1 (insulin-dependent) diabetes

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The therapy of proliferative retinopathy in diabetes is problematic if optimal blood glucose control and laser coagulation have not prevented its advance. Hypophysectomized patients have less retinopathy (Houssay phenomenon) because of the absence of growth hormone (GH). Native somatostatin was unsuitable for long-term therapy because of its short half-life and side-effects. In this study we tested the effect of the long-acting somatostatin analogue SMS 201-995 on the hormone secretion of four Type 1 diabetic patients when 3×50 μg were administered subcutaneously per day over 3 days. In all patients there was a reduction of insulin requirement (mean 37%) on day 3 without deterioration of metabolic control (mean blood glucose 7.9 versus 8.5 mmol/l). GH levels dropped by 47% and somatomedin C by 25%. Glucagon, T_3, T_4, LH, FSH, cortisol and prolactin showed no significant changes. No rebound phenomenon and no serious side-effects were observed. Our results show that raised GH and somatomedin C levels in Type 1 diabetes can be lowered selectively by SMS 201-995. Peripheral hyperinsulinism is reduced by the reduction in insulin requirement. Long-term therapy with SMS 201-995 could therefore be helpful in the treatment of diabetic macroangiopathy and microangiopathy.

420. Decrease of vipergic nerves in diabetic impotence in man

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A number of morphological and physiological studies have suggested that the potent vasodilator peptide, vasoactive intestinal polypeptide (VIP), may be the principal neurotransmitter involved in the control of penile erection. Biopsy specimens of erectile tissue from penes of men impotent due to diabetes ($n=12$), or to other causes including trauma ($n=4$), atherosclerosis ($n=4$), priapism ($n=1$) and psychogenic disorders ($n=2$) were studied by immunocytochemistry and radioimmunoassay with antiserum to VIP. It was found that the VIP-immunoreactive nerves were markedly decreased in number in the impotent specimens as compared with eight control (potent) cases. Similar changes were detected by radioimmunoassay by which the

potent group showed a VIP level of 189.9 ± 45.9 pmol/g (mean ± SEM), and the diabetic group 33.7 ± 6.7 pmol/g. The decrease, however, was not confined to diabetic impotence but was also found in impotence due to other causes, and was largely proportional to the severity of the dysfunction.

421. Decreased glucose-induced insulin release and biosynthesis by islets of rats with non-insulin-dependent diabetes: effect of tissue culture

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Non-insulin-dependent diabetes was obtained in adult Wistar rats following a neonatal streptozotocin injection. Rats with non-insulin-dependent diabetes exhibited modestly elevated plasma glucose levels (9 mmol/l), impaired glucose tolerance and a very low insulin response in vivo after a glucose load. The relationships between insulin biosynthesis, storage and release were studied in islets isolated from such rats. Since islet size was reduced in the diabetic rats, all the parameters measured were expressed on the basis of islet DNA. In diabetic rats islet, insulin content was lower (75%); islet insulin release was 60% and 48% of controls at 2.8 and 16.7 mmol/l glucose, respectively. Proinsulin biosynthesis, estimated by incorporation of ^{3}H-phenylalanine into immunoprecipitable material, was significantly higher (50%) at glucose (2.8 mmol/l), but elevation by glucose (16.5 mmol/l) was less pronounced than in control islets. However, after maintenance in tissue culture (5.5 mmol/l glucose) for 5 days, islets of diabetic and control rats were found to be similar concerning insulin content, insulin release and proinsulin biosynthesis either measured in basal or stimulated states. This may suggest that in rats with non-insulin-dependent diabetes the derangement of glucose-response is not intrinsic to the islets since it can be reversed by changing environmental conditions.

422. Peripheral versus intraperitoneal insulin administration in man

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The aim of this study was to compare insulin kinetics after intramuscular (IM), subcutaneus (SC) and intraperitoneal (IP) bolus administration in normal subjects and insulin kinetics and metabolic activity during chronic SC and IP administration in Type I (insulin-dependent) diabetes. Seven normal subjects received a bolus of 0.2 IU/kg IM, SC and IP (through a fine catheter placed into the peritoneum). IP insulin gave a sharp rise of venous free insulin peaking at 15 min (93 ± 18 mU/l); IM and SC peaked at 75 min (49 ± 5 and 50 ± 6 mU/l, respectively, $p < 0.05$ versus IP). Two Type I diabetic patients received sequentially a 15 day SC and IP continuous insulin infusion and six-point metabolic profiles were compared. Mean blood glucose was 8.1 ± 0.8 SC versus 7.3 ± 0.4 mmol/l IP. IP yielded lower mean free insulin (31.5 ± 2.9 versus 48.9 ± 6.8 mU/l, $p < 0.01$) and β-OH-butyrate (41.5 ± 7.1 versus 73.6 ± 7.7 μmol/l, $p < 0.005$), and higher glycerol (56.9 ± 7.7 versus 33.6 ± 6.3 μmol/l, $p < 0.001$) than SC insulin. In conclusion, IP bolus insulin in man has a different effect than SC or IM, peaking faster and yielding higher free insulin levels; with comparable glycaemic control, two Type I diabetic patients had lower peripheral free insulin and ketones and higher glycerol during IP than during SC administration of insulin.

423. Activated T lymphocytes, HLA-DR types and the susceptibility to Type 1 (insulin-dependent) diabetes

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Evidence has been accumulated that activated T lymphocytes are present at diagnosis in the peripheral blood of patients with Type 1 diabetes. We have evaluated the presence of activated T lymphocytes in two groups of subjects: 58 first degree relatives of Type 1 diabetic probands (25 with HLA-DR3) having two, one or no HLA haplotypes in common with the diabetic patients (group A) and 17 normal subjects (four with HLA-DR3) with no family history of diabetes or autoimmune diseases (group B). To characterize activated T lymphocytes, monoclonal antibodies (4F2 and anti-TAC) were used. In group A, blood was taken every 4 months for 18 months (214 observations), whereas in group B samples were obtained weekly for 3 months (204 observations). In group A, 22 subjects (14 with HLA-DR3) showed activated T cells with the 4F2 antibody and 11 subjects (9 with HLA-DR3) with the anti-TAC antibody. There was no correlation between activated T cells and the HLA haplotype shared with the diabetic patients. Activated T cells (both 4F2- and TAC-positive) were also found in three subjects of group B (two with HLA-DR3, one with HLA-DR6). These results suggest that activated T lymphocytes occur more frequently in subjects who have HLA-DR3, irrespective of their genetic susceptibility to Type 1 diabetes.

424. Effect of metformin on peripheral insulin resistance in Type 2 (non-insulin-dependent) diabetes mellitus

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The hypoglycaemic action of metformin is at least partially attributed to improvement of peripheral glucose utilisation in Type 2 diabetes. To test this hypothesis, peripheral insulin sensitivity was evaluated in 15 patients with Type 2 diabetes before (A) and after 4 weeks of metformin (1700 mg/day) therapy (B) by means of the hyperinsulinaemic clamp-technique (steady-state plasma insulin: 115 mU/l, plasma glucose: 8.3 mmol/l). Furthermore, insulin binding to monocytes was compared between A and B. Drug adherence was controlled by measurement of metformin plasma levels at B. Results: diabetic control, evaluated by fasting blood glucose (A: 13.3 mmol/l, B: 8.9 mmol/l, $p < 0.01$) and by glycosylated haemoglobin (A: 8.9%, B: 7.3%, $p < 0.01$), was significantly improved by metformin therapy. Insulin binding to monocytes was not significantly different between A and B (4.53 ± 0.9% versus 5.02 ± 1.0% at insulin tracer concentration). Peripheral glucose utilisation improved slightly, but significantly, after metformin therapy (A: 4.08 ± 0.51 versus B: 4.87 ± 0.62 mg/kg body weight per min; $p < 0.01$). The present data indicate that the glucose-lowering potency of metformin is at least partially caused by a positive influence on tissue sensitivity to insulin which seems to be located at the post-receptor site.

425. EEG changes during hypoglycaemia in Type 1 (insulin-dependent) diabetic patients

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The aim was to find a glycaemic threshold for EEG changes during gradual lowering of blood glucose (BG) to hypoglycaemic levels in nine fasting, recumbent, alert Type 1 diabetic patients [mean age 28 years (20–40 years), duration of diabetes 12 years (3–28 years)]. EEG was evaluated by period analysis while BG fell from 6.0 to 1.7 mmol/l (median) during an intravenous insulin infusion, after spontaneous BG increase to 2.7 mmol/l, and after intravenous glucose (BG = 5.4 mmol/l). No EEG changes were seen during the BG decrease from 6 to 2.2 mmol/l. At BG = 1.9 mmol/l, theta activity increased concomitantly with decreased alpha activity ($p < 0.025$). This was accentuated at BG = 1.7 mmol/l, where delta activity also appeared ($p < 0.025$). These changes were less prominent as BG increased from 2.0 to 2.7 mmol/l and almost disappeared after glucose administration. No inter-hemispheric differences were observed. Bisynchronous bursts of 2–4 Hz activity were seen frontotemporally in half of the patients at BG < 2 mmol/l. Reaction time was prolonged in all patients at BG < 2.2 mmol/l and still after glucose (both $p < 0.05$). All revealed signs of hypoglycaemia at BG < 2 mmol/l. Thus, EEG slowing during hypoglycaemia, indicating neuronal dysfunction, occurs abruptly at concentrations of approximately 2 mmol/l, accentuates during additional BG reduction, and shows delayed recovery after restoration of normoglycaemia.

426. Blood lactate behaviour during an insulin tolerance test in normal, obese and Type 2 (non-insulin-dependent) diabetic subjects

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The aim of this study was to evaluate the relationship between blood glucose and lactate concentrations during an insulin tolerance test (Actrapid MC 0.02 U/kg intravenously). Such low insulin dosage is able only to suppress hepatic glucose output, without stimulating hepatic glucose uptake. Therefore the rise in blood lactate concentration is due mainly to inhibition of splanchnic uptake and can be considered as an indirect sign of inhibition of gluconeogenesis. Ten normal subjects, eight obese subjects with normal glucose tolerance and 12 Type 2 diabetic patients were studied. The K glucose during an intravenous tolerance test was significantly reduced in obese (0.99 ± 0.14; $p < 0.01$) and in Type 2 diabetic patients (0.62 ± 0.11; $p < 0.001$) compared with control subjects (1.99 ± 0.26). The maximum blood lactate increase (Δ max) was also significantly reduced in obese (0.11 ± 0.02 mmol/l; $p < 0.05$) and in Type 2 diabetic patients (0.04 ±

0.01 mmol/l; $p<0.001$) compared with control subjects (0.19 ± 0.03 mmol/l). In all subjects there was a significant positive correlation between Δmax lactate and K glucose ($r=0.86$; $p<0.001$). These findings suggest that insulin resistance (reduced K glucose) in obese and diabetic subjects is due mainly to impaired inhibition of gluconeogenesis.

427. Abnormal pituitary response to growth hormone-releasing factor in diabetes; failure to suppress with glucose and insulin

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Diabetic patients with hyperglycaemia have raised growth hormone (GH) levels which worsen diabetic control. To examine the part played by the pituitary in this vicious circle, we first studied the effect of hyperglycaemia on the GH response to growth hormone-releasing factor (GHRF-40, 0.3 μg/kg) in healthy control subjects. In five subjects, modest hyperglycaemia (8.2 ± 0.4 mmol/l) suppressed the pituitary response by over 60% compared with a saline control day (peak GH 11 ± 2 versus 27 ± 3 ng/ml, $p<0.005$). Nevertheless in 12 Type 1 (insulin-dependent) diabetic patients the response to GHRF was not suppressed (peak GH 30 ± 5 ng/ml) despite marked hyperglycaemia (15.3 ± 1.5 mmol/l). To determine whether hyperglycaemia fails to suppress the response to GHRF in diabetics because it is not accompanied by hyperinsulinaemia, six patients were re-studied during an insulin infusion (1 mU/kg per min, glucose clamped at 16 mmol/l). Despite higher insulin levels than in glucose-infused controls (88 ± 4 versus 59 ± 5 mU/l) the response to GHRF was enhanced rather than suppressed (peak GH 96 ± 29 ng/ml). In conclusion; (1) hyperglycaemia potently suppresses the response to GHRF in normal subjects, but not in diabetic patients; (2) defective pituitary suppression in diabetes is not corrected by insulin (which actually augments GH release following GHRF) and (3) impaired regulation by glucose may exacerbate deranged GH secretion in diabetes.

428. Secretion of glucagon-like immunoreactivity and leucin-enkephalin from the isolated perfused jejunum of the rat

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Enkephalins have recently been detected in the central nervous system, the gastrointestinal tract, and the endocrine pancreas. The model of the isolated perfused jejunum allows observation of the hormonal responses of the organ itself without the complex interference of the whole organism. The advantage for endocrine studies is the fact that the hormone secretion may be observed in a single organ since glucagon-like immunoreactivity (GLI) and leucin-enkephalin are produced in several organs. 15 cm of the jejunum were completely isolated, the vascular system was perfused with a tyrode solution containing 1% human albumin and 0.5% Dextran T 70, whereas the lumen was perfused with isotonic sodium chloride solution. A luminal stimulation by glucose (200 mmol/l) induces the secretion of GLI in a well-known biphasic pattern. Under these experimental conditions, the secretion of leucin-enkephalin is inhibited during the first 10 min corresponding to the first peak of GLI-release. However, afterwards secretion is induced to 1.35 ng/ml. Stimulation by arginine (20 mmol/l) induced a lower but simular response. In conclusion the isolated perfused jejunum, luminal application of glucose or arginine induces biphasic GLI secretion and suppresses the first phase of leucin-enkephalin secretion. Thus enkephalins may provide an important link between endocrine secretion and metabolism.

429. Polyphosphoinositide metabolism in rat islets of Langerhans

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Recent evidence suggests that an early event in the stimulus-secretion coupling mechanism of a variety of secretory cells is the rapid breakdown of inositol containing phospholipids, particularly the polyphosphoinositides. In this study we have investigated the effects of glucose and carbachol on the metabolism of polyphosphoinositides in rat islets of Langerhans. Rat islets were prelabelled for 90 min with ^{3}H-inositol and the free inositol was removed from the medium. The islets were then stimulated for 30, 60 and 120 s with glucose (20 mmol/l) or carbachol (1 mmol/l) and the radioactivity in phosphatidylinositol, phosphatidylinositol 4-phosphate, phosphatidylinositol 4,5-bisphosphate, inositol phosphate, inositol 1,4-bisphosphate and inositol 1,4,5-trisphosphate was determined. Both glucose and carbachol induced a rapid decrease in the radioactivity associated with phosphatidylinositol 4,5-bis-phosphate and phosphatidylinositol 4-phosphate and a corresponding increase in the radioactivity associated with inositol 1,4,5-trisphosphate and inositol, 1,4-bisphosphate. These observations are compatible with the hypothesis that an early event in stimulus-secretion coupling in the β cell is the rapid breakdown of polyphosphoinositides.

430. Metformin potentiation of insulin action in soleus muscle of streptozotocin-diabetic mice

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Metformin reduces hyperglycaemia in non-insulin-dependent diabetes mellitus without increasing insulin concentrations. To investigate the mechanisms responsible, glucose uptake and metabolism were examined in soleus muscles isolated from streptozotocin (200 mg/kg) diabetic and normal (non-diabetic) mice treated with metformin ($250\ mg\cdot kg^{-1}\cdot day^{-1}$) for 3 weeks. In streptozotocin-diabetic mice the severity of hyperglycaemia was reduced (60%) by this dose of metformin. Uptake of ^{14}C-3-0-methylglucose into soleus muscle was increased by metformin at submaximally- and maximally-stimulating insulin concentrations (by 34% and 8% respectively). Metformin increased (by about 15%) the oxidation of U-^{14}C-glucose to $^{14}CO_2$ at insulin concentrations of 100 mU/l and above. The conversion of U-^{14}C-glucose to ^{14}C-glycogen was also increased (by about 30%) by metformin at these insulin concentrations. In normal mice, glucose oxidation in soleus muscle was not significantly altered by metformin, although glycogenesis was increased by the drug with maximal insulin stimulation. Metformin did not significantly increase lactate formation in either streptozotocin-diabetic or normal mice. The results demonstrate that the hypoglycaemic effect of metformin in streptozotocin-diabetic mice is associated with increased glucose uptake, glycogenesis and glucose oxidation in soleus muscle at submaximally- and maximally-stimulating insulin concentrations.

431. Hypertension and mortality in diabetic and non-diabetic Finnish men

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We studied the association of hypertension and 6-year total and cardiovascular disease (CVD) mortality among 139 diabetic and 8,900 non-diabetic men, aged 40–69 years, who were initially CVD-free. These men belonged to total populations or random samples of populations examined in various parts of Finland in 1966–1972. Diabetes was diagnosed on the basis of blood glucose or history of treatment. Twenty-three diabetic men and 608 non-diabetic men died during follow-up. A hypertensive group (systolic blood pressure $\geqslant$ 160 mmHg and diastolic blood pressure $\geqslant$ 95 mmHg on drug treatment) and a normotensive group were formed. The multiple logistic model was used in data analysis. In comparison with normotensive non-diabetic subjects the age-adjusted relative risk (RR) of death was 1.9 in hypertensive non-diabetic and 3.0 in hypertensive diabetic patients. Corresponding RRs for CVD death were 2.6 and 4.7. A comparison of hypertensive non-diabetic with normotensive non-diabetic subjects and hypertensive diabetic with normotensive diabetic subjects suggested that hypertension was a similar or stronger risk factor for CVD death in diabetic than non-diabetic subjects. Adjustment for smoking and serum cholesterol resulted in higher RRs.

432. Anti-islet immunity and T cell subsets in Type 1 (insulin-dependent) diabetes

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Type 1 diabetes can be associated with anti-islet immunity and anomalies in T-lymphocyte subsets, but their respective pattern and time course remain controversial. In 56 patients and 20 controls, peripheral lymphocyte subsets were enumerated using the OKT monoclonal sera. Anti-islet immunity was assessed by: (1) the capability of lymphocytes to inhibit selectively the response of normal murine β cells to stimuli in vitro; (2) the complement-dependent cytotoxicity of sera to ^{51}Cr-labelled islet cells; (3) the potency of sera to inhibit selectively the β-cell response to stimuli in vitro. In all newly diagnosed patients (< 30 days; $n=18$), the OKT4 (helper) and OKT8 (suppressor-cytotoxic) cell counts declined, while OKT3$^+$ cell counts remained normal. The OKT8$^+$ subset was particularly affected: the mean OKT4/OKT8 ratio was higher than normal. Later on, a marked depletion in all T cell subsets developed: the OKT4/OKT8 ratio fell within or below normal, with few exceptions. Lymphocytes from most newly diagnosed patients (13/18), from all children ($n=13$) and all subjects with asso-

ciated autoimmune manifestations ($n=12$) suppressed insulin release in vitro. Complement-dependent cytotoxicity of sera and their insulin-suppressive potency were detected in 50% of patients, whatever their age, clinical context or duration of the disease.

433. Fractional and total hepatic extraction of glucose during intraduodenal glucose loading in conscious swine

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Hepatic extraction of glucose has frequently been reported to increase during the disposal of ingested (versus intravenous glucose). Our previous results indicate the formation of identical amounts of hepatic glycogen during oral and intravenous glucose loading. We therefore infused 20% glucose (C^{14}-labelled) intraduodenally into four conscious 24-h fasted pigs with the concurrent intravenous infusion of H^3-3-glucose and indocyanine green (ICG). Labelled and unlabelled glucose, tracer and ICG concentrations were measured in arterial, portal and hepatic venous plasma. Total liver plasma flow was calculated from the ICG levels, and was assumed to be 80% portal. From the fluxes of glucose and tracer into and out of the gut, liver and splanchnic bed the following quantities were calculated: Absorption of (C^{14}) glucose was $73 \pm 8\%$ during the period studied, whereas net splanchnic uptake was $53 \pm 8\%$ leading to a hepatic uptake of $29 \pm 5\%$ relative to the absorbed load. Fractional extractions (extraction: influx) of H^3-3-glucose by the gut and by the liver were $3.5 \pm 0.4\%$ and $3.4 \pm 0.7\%$, respectively. The latter quantity was identical with the fractional extraction calculated using unlabelled glucose ($3.9 \pm 0.7\%$) and to the basal rate ($3.6 \pm 1.3\%$) and yielded a hepatic uptake (fractional extraction × glucose flux into liver) of 32.9% of the absorbed glucose. In conclusion, both the liver and the gut took up about 3–4% of the glucose presented to them. This rate does not change significantly during a glucose load, but is consistent with the total hepatic uptake of glucose equivalent to 30% of the intraduodenal load – a quantity similar to the amount of glycogen formed.

434. Glucose tolerance, insulin receptors and insulin resistance in uraemic patients: effects of chronic haemodialysis

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Insulin resistance (IR) is one of the main factors responsible for impaired glucose tolerance, being frequently found in uraemic patients. However, its cause is still unclear. In this study, we have studied glucose tolerance, IR and insulin receptors in 12 uraemic patients, before and after 1 month of haemodialysis. IR was evaluated infusing glucose (23.3 mmol/min), insulin ($0.77\ mU \cdot kg^{-1} \cdot min^{-1}$) and somatostatin (500 µg/h). Since similar steady state plasma insulin concentration was maintained in all subjects, the steady state plasma glucose (SSPG) was considered an index of IR. Insulin receptors were studied on peripheral blood monocytes and erythrocytes. Glucose tolerance was evaluated by an oral glucose tolerance test. Uraemic patients showed higher mean blood glucose (52.6 ± 6.4 versus 28.3 ± 3.5 mmol/l; $p<0.001$) and, furthermore, IR demonstrated by higher SSPG values (7.2 ± 0.4 versus 4.8 ± 0.3 mmol/l; $p<0.05$). These metabolic alterations were significantly correlated ($r=0.64$; $p<0.05$) and disappeared after 1 month of haemodialysis (mean blood glucose 52.6 ± 6.4 before versus 32.7 ± 4.1 mmol/l after; $p<0.01$; SSPG 7.2 ± 0.4 before versus 5 ± 0.5 mmol/l after; $p<0.05$). Insulin bound was not significantly different from controls (1.84 ± 0.1 versus 2.1 ± 0.09) and was not modified after haemodialysis (1.88 ± 0.1). We conclude that IR in uraemia is the consequence of non-receptor factors, reversible after haemodialysis.

435. Ultrastructural and immunohistochemical quantitative study of the endocrine pancreas in human diabetes secondary to haemochromatosis

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The morphology of the endocrine pancreas in diabetes secondary to haemochromatosis is poorly documented. Insulin (B), glucagon (A), somatostatin (D) and pancreatic polypeptide (PP) cells were quantitated after immunoperoxidase staining and examined by electron microscopy in the pancreas of eight controls and six patients with haemochromatosis. Four suffered from insulin-requiring diabetes and two had glucose intolerance. The total weight of pancreatic parenchyma was reduced by about 50% in the patients. Iron overload was much greater in exocrine than in endocrine tissue. The general aspect of the islets was normal. However, in the four diabetic patients the total mass of B cells was dramatically lowered. The mass of D cells was also consistently decreased, that of A cells was either decreased or normal and that of PP cells was unchanged. In the two patients with glucose intolerance the mass of the four cell types was within normal range. In all diabetic patients, electron microscopy revealed poorly granulated B cells or B cells with numerous empty granular sacs or pale granules. Abnormalities of the rough endoplasmic reticulum were also evident. In conclusion, this study has characterized the alterations of the endocrine pancreas in haemochromatosis and shows that these abnormalities are distinct from those observed in primary diabetes.

436. Thermic effect of oral glucose in Graves's disease and in experimental hyperthyroidism in man

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The rates of glucose and lipid oxidation and the total energy expenditure were measured by means of indirect calorimetry in the post-absorptive state and during the 3 h following the ingestion of a 100 g glucose load in seven patients with Graves's disease before and after treatment and in eight healthy subjects after administration of either triiodothyronine (T_3, 125 µg/day) or a placebo for 7 days. In the two hyperthyroid groups, basal lipid oxidation was markedly elevated while basal glucose oxidation was not different from their own controls. After glucose ingestion, suprabasal glucose oxidation was significantly increased in hyperthyroid patients (36 ± 3 versus 19 ± 2 g/3 h, $p< 0.001$) and in T_3-treated subjects (22 ± 2 versus 16 ± 1 g/3 h, $p< 0.05$), while lipid oxidation decreased to control values after 60 min in both hyperthyroid groups. The thermic effect of the load, expressed as the mean percentage increase in resting metabolic rate over 3 h, did not differ from their own controls in hyperthyroid patients (12 ± 2 versus $9 \pm 2\%$) and in T_3-treated subjects (12 ± 1 versus $10.1 \pm 0.7\%$). It is concluded that the high energy expenditure in hyperthyroid states does not result from an increased carbohydrate induced thermogenesis.

437. Effects of a new long-acting α-glucosidase inhibitor (Bay-1248) on glycaemic control in insulin-dependent diabetes mellitus

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α-Glucosidase inhibition improves glucose tolerance and decreases insulin requirements in patients with insulin-dependent diabetes mellitus. The present studies were undertaken to assess the potential of a new long-acting α-glucosidase inhibitor as an adjunct to insulin in the treatment of diabetes, using a closed-loop insulin infusion device (Biostator). Six insulin-dependent diabetic patients were connected to the Biostator for 24 h on two occasions, to obtain blood glucose profiles and assess insulin requirements during ingestion of standardized diet (30 Kcal/kg, carbohydrate 45%, fat 35%, protein 20%) following single administration of Bay-1248 (20 mg, p.o.) or placebo, prior to breakfast (double-blind experiments). Bay-1248 reduced meal-related blood glucose increases by 15–20% ($p<0.05$) and decreased insulin requirements for each meal (18 ± 3 versus 13 ± 3 U, 18 ± 3 versus 14 ± 2 U, 19 ± 3 versus 15 ± 3 U for breakfast, lunch and dinner, respectively, $p<0.05$). Bay-1248 delayed meal absorption and reduced abrupt increases or decreases in blood glucose concentrations. Symptoms related to its use were absent. In conclusion, Bay-1248 renders insulin more effective in controlling diabetic hyperglycaemia. This insulin-sparing effect and the smoothening of blood glucose profile may make this inhibitor a useful adjunct to insulin in the treatment of insulin-dependent diabetes.

438. Changes in macula densa of the juxtaglomerular apparatus in experimental diabetes

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Glomerular changes have been extensively studied in experimental diabetes. However, alterations in the juxtaglomerular apparatus have never been investigated systematically. Macula densa cells of the juxtaglomerular apparatus were studied by light and electron microscopy in five streptozotocin-diabetic and five control rats. The animals were kept diabetic for 50 days with blood glucose levels of 22 ± 1 mmol/l (mean ± SD). Pronounced alterations were seen in the macula densa regions of the diabetic rats. Light microscopy of Epon-embedded tissue showed that the cells contained a strongly PAS-positive material

which could be digested with diastase. Electron microscopy revealed that the cells had an intact cell membrane and nucleus. The cytoplasm of the abnormal cells contained strikingly few organelles mostly located close to the nucleus or to the cell membrane. The majority of the remaining organelles were mitochondria. The cytoplasm of the abnormal cells were loaded with 20–35 nm large, diffusely distributed granules resembling glycogen particles. 25 well-defined juxtaglomerular regions were located on paraffin embedded PAS-stained sections. In the diabetic group abnormal PAS-positive cells were found in 35% of the macula densa but never in the control animals. The changes in macula densa in the diabetic animals indicate that glomerulo-tubular feed-back mechanism might be disturbed.

439. Absence of a thermogenic defect in response to glucose/insulin infusions in human obesity

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The thermogenic effect of glucose (TEG) is due to the metabolic processing of the ingested or infused glucose and to a sympathetically mediated component. To investigate whether the decreased TEG in obese individuals is 'real' or due to their insulin resistance and hence lower glucose uptake and storage rates, we studied the metabolic rate in nine obese (2 male, 7 female; 75 kg; 33% fat) and six control subjects (3 male, 3 female; 62 kg; 19% fat) during infusion of the same amount of glucose. After a 45-min baseline, 20% glucose was infused for 195 min at 609 mg/min and euglycaemia was maintained by adjusting the insulin infusion rate. At 2 h, propranolol was infused (bolus 100 μg/kg, 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) for the remaining 75 min. During glucose infusion without propranolol the metabolic rate rose from 1.10 ± 0.07 to 1.24 ± 0.07 (controls) and from 1.16 ± 0.08 to 1.28 ± 0.08 kcal/min (obese) with similar rates ($\simeq 360$ mg/min) and similar costs of glucose storage (0.39 kcal/g). In both groups the metabolic rate fell significantly to 1.20 ± 0.07 (controls) and 1.21 ± 0.07 kcal/min (obese) with propranolol. In conclusion, when comparable amounts of glucose enter the tissue, TEG is similar in obese and control subjects. The observed sympathetically-mediated component of TEG was more variable in the obese than the lean.

440. Vascular responses to postural change in the diabetic neuropathic foot

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In normal subjects foot blood flow falls by $87.4 \pm 12.4\%$ (mean ± SD) on standing, thereby limiting hyperfiltration. As this response is believed to represent an axonal reflex, we studied diabetic patients with peripheral neuropathy (PN). Eleven diabetic patients with PN (absent ankle jerks and impaired vibration sense by biothesiometry) and no evidence of peripheral vascular disease were compared with 11 pair-matched healthy control subjects. Foot skin blood flow was determined by laser doppler flowmetry (1) with the foot at heart level, and (2) with the foot 50 cm below the heart with the subject lying down on a specially constructed couch. In normal subjects, foot blood flow fell by $73.2 \pm 15.6\%$ on dependency, compared with $53.6 \pm 19.3\%$ ($p < 0.05$) in patients with PN. Thus the vascular response to postural change is impaired in the diabetic neuropathic foot. It is likely this failure to increase pre-capillary resistance results in raised capillary pressure which may be one aetiological factor in the development of neuropathic oedema.

441. Is the insulin resistance of patients with Type 2 (non-insulin-dependent) diabetes mellitus secondary to insulin deficiency?

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Defects in insulin secretion and action have been documented in patients with Type 2 diabetes, leading to the suggestion that both fasting hyperglycaemia and insulin resistance are secondary to insulin deficiency. To test this hypothesis, insulin secretion (plasma insulin response to oral glucose) and insulin action (insulin clamp) were determined in 25 patients with Type 2 diabetes. Relationships existed between incremental plasma insulin response to glucose and degree of fasting hyperglycaemia ($r = -0.45$, $p < 0.05$) and insulin-stimulated glucose utilization ($r = 0.25$, NS). Consequently, differences in insulin secretory response accounted for only 20% of the variance in fasting plasma glucose level and 6% of the variance in insulin resistance in Type 2 diabetes. In contrast, the relationship between insulin action and fasting hyperglycaemia was highly correlated ($r = 0.79$, $p < 0.001$), indicating that differences in insulin action accounted for about 60% of the variance in these patients. In conclusion, differences in insulin-secretory response contribute modestly to magnitude of glycaemia and not at all to variations in insulin resistance in Type 2 diabetes, permitting rejection of the hypothesis that insulin resistance is secondary to insulin deficiency. Furthermore, it appears that differences in insulin action play a greater role than differences in insulin secretion in determining degree of fasting hyperglycaemia in Type 2 diabetes.

442. IgG insulin binding prior to insulin treatment

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The immune response to injected insulin shows considerable heterogeneity among insulin-treated individuals and some fail to respond to the most immunogenic form, i.e. proinsulin-contaminated bovine insulin. Overlap between the binding values of sera from normal and insulin-treated patients is thus inevitable, but is partly contributed to by a degree of background binding inherent in the assay technique. It has recently been suggested that low levels of insulin antibody may be a marker of β-cell damage prior to the administration of insulin. To examine this possibility we have used our standard immunochemical assay in which anti-IgG is added to glass tubes containing ^{125}I-insulin + test serum and compared it with data obtained using an otherwise identical assay in which glass tubes were coated with human serum albumin to reduce the level of background binding. 112 diabetic sera, obtained immediately before insulin treatment was started, gave a mean ± SD binding value of $1.40 \pm 0.67\%$ (uncoated) and $0.70 \pm 0.27\%$ (coated), whereas 23 normal sera gave a mean binding value of $1.40 \pm 0.61\%$ (uncoated) and $0.69 \pm 0.39\%$ (coated). We conclude that exogenous insulin administration is a necessary but not always sufficient requirement for insulin antibody production in man.

443. Formation and functional characteristics of islets obtained by tissue culture of fetal rat pancreas

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Cell suspensions prepared by collagenase digestion of the pancreas of rat fetuses (21.5 days) were cultured according to Hellerström's technique in RPMI medium containing glucose (10 mmol/l). Exocrine cells disappeared rapidly, whereas endocrine cells and fibroblasts proliferated. Endocrine cells were first arranged in monolayers, but progressively detached from the bottom of the dish to form islets essentially composed of β cells with few non-β cells at the periphery. Total insulin content of dishes was maximal after 9 days, and fractional insulin release was about 20%/day. After one week, islets incubated in glucose-free Krebs buffer released < 1% of their insulin content over 2 h. Glucose (17 mmol/l) caused a slower and weaker (threefold) stimulation than leucine (10 mmol/l) or arginine (3–5-fold). The effect of glucose was potentiated by theophylline, and that of the amino-acids by theophylline or low glucose (5.6 mmol/l). Diazoxide, which inhibits insulin release from adult β cells by increasing K-permeability, decreased the response to glucose and leucine (which lower K-permeability), but not to arginine (which depolarizes by another mechanism). The fetal character of these β cells thus remains evident even after one week of culture, but it is apparently not due to abnormal K-permeability of the plasma membrane.

444. Impact of risk factors on coronary mortality in diabetic and non-diabetic subjects

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Total, cardiovascular and coronary mortality were investigated in a prospective population study comprising 12,572 men and 12,300 women, aged 40–69 years at entry. 273 of the men and 292 of the women had diabetes at the initial investigation. In a mean follow-up time of 10 years, 2,485 of the men and 1,140 of the women had died. 1,082 of the deceased men and 334 of the women died from coronary heart disease. The age-adjusted coronary mortality risk of diabetic patients was threefold compared with non-diabetic subject. The impact of risk factors on coronary mortality was investigated by the Cox model. Age and smoking had almost significant interaction with diabetes. Smoking appeared to have a significantly smaller impact on mortality in diabetic than in non-diabetic subjects. Hypertension and

hypercholesterolaemia increased coronary mortality risks similarly in diabetic and non-diabetic subjects. Adjustment for other risk factors did not change the mortality risk in diabetes. The results suggest that, although diabetes is a significant independent risk factor for coronary mortality, hypertension and hypercholesterolaemia increase the mortality risk of diabetic patients similarly as in non-diabetic subjects.

445. Selective inhibition of glucose-induced proinsulin biosynthesis by 2′, 5′-oligoadenylate in mouse pancreatic islets

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Synthesis of 2′, 5′-oligoadenylate (2–5 A) from ATP can be induced in most types of mammalian cells by interferon. 2–5 A acts as a secondary messenger of interferon in inhibiting new protein synthesis by activating a 2–5 A-dependent endoribonuclease, which digests free cytoplasmic RNA (including some mRNA) in the cell. We have investigated the effect of a non-phosphorylated analogue of 2–5 A, known as 2–5 S 'core', on isolated mouse pancreatic islets. 2–5 A 'core' can enter cells and inhibit protein synthesis. Exogenous concentrations of 2–5 A 'core' above 1 μmol/l specifically inhibited biosynthesis of proinsulin induced by glucose 20 mmol/l ($p<0.01$) within 2 h. A lesser inhibition was observed for total protein synthesis, with no effect on insulin secretion. Similar observations have been made with mouse islets incubated with interferon. Indeed, we have also shown in mouse islets incubated with interferon that 2–5 A-dependent endoribonuclease activity was detectable at 2–5 A (5 nmol/l). It is concluded that free mRNA in the cytoplasm of β cells is necessary for the full stimulation of proinsulin synthesis by glucose.

446. Subcutaneous absorption of ^{65}Zn- and ^{125}I-labelled neutral insulin solutions

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The purpose was to investigate the absorption of neutral zinc insulin solutions, in which the insulin molecules are mainly associated as hexamers with 2 Zn^{2+} per hexamer. A 14-mono-^{125}I insulin and ^{65}Zn were used for preparation of human, porcine and bovine Actrapid preparations. The three insulins and a solution of Zn^{2+} alone were administered subcutaneously according to a randomized scheme to 22 pigs, each tested twice (Danish Landrace x Yorkshire, female, body weight approximately 60 kg, insulin dose 0.2 U/kg). The radioactivity from each isotope at the subcutaneous depot was assessed by external γ-counting. The disappearance of ^{125}I-insulin was approximately monoexponential, whereas that of ^{65}Zn could be resolved into at least two phases. The disappearance of both tracers was significantly correlated and no differences in absorption between the three insulin species were found. The mean initial rates of disappearance of ^{65}Zn and ^{125}I-insulin were 37 and 41%/h, respectively ($p<0.001$), while that of ^{65}Zn without insulin was higher (61%/h; $p<0.001$). Five hours after injection 30% of the ^{65}Zn, but only 10% of the ^{125}I-insulin remained ($p<0.001$). It is concluded that the zinc insulin hexamer complex dissociates partially or completely before absorption. The quantitative aspects of these results have been corroborated by pharmacokinetic modelling.

447. Effects of postaglandin E_2 on glucose metabolism in human adipocytes: interaction with insulin

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Recently we have described that prostaglandin E_2 (PGE_2) binds to specific receptors on human adipocytes. The anti-lipolytic effect of PGE_2 seems to be mediated by this binding. To investigate whether PGE_2 also has effects on glucose metabolism, we studied the effect of PGE_2 on glucose transport, glucose oxidation and lipogenesis in human adipocytes. PGE_2 stimulated glucose metabolism in a dose-dependent manner with half-maximal stimulatory effect at 0.7–4 nmol/l and maximal effect at 1 μmol/l of PGE_2. The maximal stimulation of glucose transport measured by 3-0-methyl-glucose was 50% above basal level (from 4.1 to 6.2 pmol/10^5 cells per 8 s), the maximal stimulation of glucose oxidation measured by ^{14}C-glucose was 60% above basal level (from 5.5 to 8.8 pmol/10^5 cells per 90 min), and the maximal stimulation of lipogenesis was 34% above basal level (from 50 to 68 pmol/10^5 cells per 90 min). When insulin and PGE_2 were added together the effect of PGE_2 on lipogenesis was additive for all insulin concentrations tested, whereas the effect of PGE_2 on glucose oxidation was only additive for submaximal insulin concentrations tested. These findings indicate that PGE_2 mediates its effects through other pathways than insulin. In conclusion, PGE_2 had a significant stimulatory effect on glucose metabolism in human adipocytes and this effect is probably mediated through the binding between PGE_2 and its specific receptor.

448. Insulin receptor phosphorylation in human monocytes

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Human monocytes are widely used to study insulin receptor binding and its rôle in the pathogenesis of insulin resistance in man. Recent evidence suggests that the insulin receptor contains tyrosine-kinase activity which might be important for the signalling function of the insulin receptor. We describe here that determination of monocyte receptor kinase activity is possible from 60 ml blood. Monocytes were isolated by Ficoll Hypaque gradient. Cells were disrupted by repeated freezing and thawing in the presence of protease inhibitors. The receptor was solubilized by Triton X-100 and was enriched by wheat-germ affinity chromatography. Phosphorylation was studied with γ-[^{32}P]ATP and insulin, and the receptor was identified by immunoprecipitation with receptor antibody, polyacrylamide gel electrophoresis and autoradiography. The receptor bands were cut from the gel and incorporation of [^{32}P] was counted. Insulin stimulated receptor phosphorylation two- to threefold.

449. Severe life events and their relationship to the aetiology of Type 1 (insulin-dependent) diabetes

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In genetically susceptible individuals, it is possible that stressful life events may act as precipitating factors for Type 1 diabetes. Using the Brown and Harris Life Events and Difficulties Schedule, we interviewed 11 newly diagnosed diabetic patients and their sibling controls (aged 17–34 years), participating in the Barts-Windsor-Middlesex prospective family study. Of the diabetic-sibling pairs, six were HLA identical and four haploidentical; eight were islet cell antibody (ICA) IgG-positive and four were ICA complement fixing-positive. For the 3 years preceding the clinical diagnosis of diabetes, severe events were rated for the diabetic-sibling pairs. Over the 6 months prior to diagnosis, 46% of diabetics had experienced at least one severe event and 18% had more than two. Proportions for siblings were much less being 18% and 0%, respectively. Over the 2½ year period prior to diagnosis, 73% of diabetics had one or more severe events and 55% had more than two compared with 36% and 9%, respectively for siblings. These preliminary results suggest that stressful life events may be one of the several environmental factors possibly involved in the aetiology of Type 1 diabetes.

450. Plasma lipid fatty acids and platelet function with continuous subcutaneous insulin infusion in Type I (insulin-dependent) diabetes mellitus

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Thirteen fairly well controlled Type 1 diabetic patients (HbA_1 9.7 ± 0.5%) were consecutively submitted to two 6 week test periods of: (1) continuous subcutaneous insulin infusion (CSII) and (2) conventional insulin therapy. Plasma lipids, fatty acids in plasma lipids and platelet function were estimated at baseline and at the end of each test period. In six patients (group A), CSII produced a significant ($p<0.02$) decline of HbA_1 from a baseline value of 10.5 ± 0.6% to 8.9 + 0.5%. No change was observed in the remaining seven (group B). In both groups, plasma lipids and apoproteins remained unchanged at the end of the two test periods compared with baseline. In group A, CSII was followed by a significant increase of arachidonate in plasma lipids (4.1 ± 0.9 versus 5.6 ± 0.8%, $p<0.01$) and by improvement of platelet aggregation in vitro (1123 ± 136 versus 671 ± 157 cm^2, $p<0.05$) and of thromboxane B_2 released from platelets (54.9 + 19.1 versus 15.5 ± 5.4 pmol/10^8, $p<0.1$). No changes were observed in group A under conventional therapy, and in group B during the two test periods. These results indicate that, even in fairly well controlled diabetic patients, CSII can aid in stimulating the biosynthesis of arachidonate, the metabolic precursor of prostaglandins of the two series, and in turn improve platelet function.

451. Amino-acid mixture-induced pancreatic glucagon secretion is increased in hypothalamic obesity

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The concept that hyperinsulinaemia of various obesity syndromes (in rodents or man) is accompanied by hyperglucagonaemia is a matter of debate. The present study was an attempt at clarifying the problem of glucagon secretion in experimental obesity provoked by bilateral lesions of the ventromedial hypothalamic (VMH) area in the rat. Perfused pancreases of normal and VMH-lesioned rats were used for assessing portal levels of the hormone. Pancreases were challenged with a mixture of 20 amino-acids (AA) used either under physiological (2.5 and 5 mmol/l, with 5 mmol/l glucose) or under pharmacological conditions (15 mmol/l). When pharmacological and/or extreme (e.g. no glucose in medium) conditions were used, as has often been the case in previous studies, artefactual upward shifts of immunoreactive pancreatic glucagon secretion were observed that equalized the hormonal output in normal and VMH-lesioned animals. However, when experimental conditions were close to physiological ones, i.e. either mimicking pre-prandial aminoacidaemia (2.5 mmol/l AA) or the aminoacidaemia that follows ingestion of a mixed meal (5 mmol/l AA), pancreases from VMH-lesioned rats oversecreted glucagon by 2–3-fold when compared with controls. This strongly suggests that hypothalamic obese animals hypersecrete not only insulin but also glucagon, a likely explanation for their normal glycaemia.

452. Factors related to improved metabolic control in Type 2 (non-insulin-dependent) diabetic patients during long-term physical exercise: a controlled randomized study

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The effect of 4 months' physical exercise was studied in 25 Type 2 diabetic patients (aged 45–60 years), divided into active and control groups. The possible anti-diabetic medication was unchanged. Patients had 4–7 weekly 30-min periods of walking or jogging at 70% intensity of maximal aerobic capacity (VO_2 max). Their pre- and post-exercise values were: VO_2 max 26.7 and 29.3 $ml \cdot min^{-1} \cdot kg^{-1}$ ($p < 0.001$), body weight 85.2 and 83.0 kg ($p < 0.05$), fasting plasma glucose 11.8 and 10.5 mmol/l (NS), glycosylated haemoglobin A_1 9.5 and 8.6% ($p < 0.02$), 2-h plasma glucose during an oral glucose tolerance test 19.6 and 16.5 mmol/l ($p < 0.01$), fasting plasma insulin 17.3 and 15.4 mU/l (NS). The response of plasma insulin and C-peptide after the glucose tolerance test increased significantly: 2-h plasma C-peptide was 1.24 before and 1.65 nmol/l after exercise ($p < 0.01$). In control subjects all values remained unchanged. Patients benefiting most from the exercise had: increase in VO_2 max > 10%; decrease in body weight > 1.5 kg; initial fasting plasma glucose < 13 mmol/l and plasma insulin > 10 mU/l; fasting plasma C-peptide > 0.5 nmol/l and plasma C-peptide after intravenous glucagon > 1.5 nmol/l. Long-term physical exercise improves the metabolic control in Type 2 diabetics patients who have sufficient insulin reserves. The improvement may be explained in part by increased insulin response in addition to increased insulin sensitivity reported in earlier studies.

453. Direct evidence that glucose both reduces and increases cytosolic free Ca^{2+} in mouse pancreatic β cells

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Glucose-stimulated insulin release from pancreatic β cells is thought to be initiated by an increase of the free cytosolic Ca^{2+} ($Ca^{2+}{}_i$). This concept was tested using the intracellular Ca^{2+}-indicator quin-2 to monitor changes in $Ca^{2+}{}_i$ during glucose-stimulation. Dispersed pancreatic β cells were prepared from *ob/ob*-mice. In the presence of 1.2 mmol/l Ca^{2+} these cells had a resting Ca^{2+} of 162 ± 9 nmol/l ($n = 16$). Exposure to 20 mmol/l D-glucose gradually increased Ca^{2+} after a lag period of 1–3 min. After 5 min $Ca^{2+}{}_i$ levelled off at a value corresponding to an increase of 40%. When the entry of Ca^{2+} was inhibited by adding 50 µmol/l D-600 or lowering extracellular Ca^{2+} to 0.2 mmol/l, glucose promptly and permanently reduced $Ca^{2+}{}_i$ by 15%. Quin-2 did not affect glucose-stimulated insulin release or the metabolism of the sugar. It can therefore be concluded that the initial action of D-glucose in the pancreatic β cell is to reduce $Ca^{2+}{}_i$. This effect is subsequently superseded by an increase of $Ca^{2+}{}_i$ due to influx of Ca^{2+} through voltage-dependent channels. The studies indicate that the well known dual action of D-glucose on the efflux of radioactivity from islets loaded with ^{45}Ca is associated with concomitant changes of $Ca^{2+}{}_i$.

454. Is diabetes a Jewish disease? The Israel GOH study

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A high prevalence of Type 2 (non-insulin-dependent) diabetes in Jews has been claimed in clinic-based studies in the United States and Zimbabwe, but attributed to the purportedly higher utilization of medical services by Jews. In Israel, screening of selected population groups for post-prandial glycosuria or fasting hyperglycaemia with subsequent evaluation of positive cases, indicated a rate of zero among newly arrived Yemenites and rates > 10% in long-term Yemenite residents and other ethnic groups. We tested a nationwide sample, aged 40–70 years ($n = 2387$), equally representing all continents of origin (27 countries), by oral glucose tolerance test. The total rate of diabetes was 15.9% (10.3% previously known). Prevalence rates ranged from 13% to 23% in immigrants from 18 of 27 countries (94% of the sample). Prevalence generally increased with length of residence in Israel, but was one-third lower in those Israeli-born. Reported rates obtained with similar methodology in non-Jewish Caucasoids are about 5%. It is concluded that the prevalence of diabetes in Jews of all ethnic groups is high. The relative genetic and environmental contributions remain to be determined.

455. The Canon CR2-45NM retinal camera markedly improves the detection of diabetic retinopathy through undilated pupils

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In view of the difficulties with obtaining adequate ophthalmoscopy in busy diabetic clinics, we have assessed the potential of the new Canon CR2-45NM polaroid non-mydriatic retinal camera in these clinics. Patients were selected randomly. Those already attending an ophthalmologist or with Type 1 (insulin-dependent) diabetes for < 10 years were excluded. Fundal photographs were taken through undilated pupils and then patients underwent conventional ophthalmoscopy through both undilated and dilated pupils. Of 137 eyes studied, 24 showed diabetic retinopathy (DR) on the photographs. In only two (8%) of these was DR detected by ophthalmoscopy through undilated pupils and in only eight (33%) was it detected through dilated pupils. The DR missed by ophthalmoscopy through undilated pupils included two eyes with large circinate exudates encroaching on the macula. 23% of photographs were unreportable because of lack of familiarity with the camera, cataracts, slow recovery of the second pupil and poor patient compliance. With further experience this failure rate is expected to decline. This easy-to-use and relatively cheap camera could prove an invaluable aid to the detection of DR in diabetic clinics.

456. In vitro non-enzymatic glycosylation of human platelet membranes

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Abnormalities of platelet functions in diabetes mellitus are well known. Since non-enzymatic glycosylation (NEG) of several proteins has been shown to determine alterations of relative functions, we tested the hypothesis that the proteins of human platelet membranes also undergo NEG in vitro. Platelet concentrates from normal volunteers were freshly prepared accordingly to blood bank procedure, and washed free of erythrocytes. The membranes were separated by pelletting (40,000 *g* for 40 min) the platelet lysate. The membrane proteins were incubated with 80 mmol/l glucose and tracer amount of ^3H-D-glucose (Sp. act. 50 mCi/mg) in phosphate buffer for 144 h at 37 °C in sterile dark conditions. As a positive control, human albumin (Behring) was incubated under the same conditions. At 0, 24, 72 and 144 h the glucose bound was determined by measuring the trichloracetic acid-precipitable radioactivity. After 24 h the glucose incorporated was 56 nmol/mg of proteins without further increase. Parallel incubations of membrane proteins and human albumin, with and without glucose at the mentioned conditions, after mild hydrolysis, were analyzed by high performance liquid chromatography. The hydrolysates of membrane proteins and human albumin with glucose showed a sharp peak which coeluted with that of 5-hydroxymethyl-2-furfuraldehyde (Sigma) used as reference. No similar peak was detected in control incubations. These results indicate that membrane proteins of human platelets undergo NEG in vitro.

457. Survival and growth of fetal human islet cells in vitro

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The success in promoting growth of human fetal islet cells in vitro has been limited. In the present investigation a method previously de-

scribed in our laboratory for preparing islets in vitro from fetal pancreas has been applied. Human pancreatic glands of different gestational age were obtained from 37 consecutive prostaglandin-induced abortions. After mild collagenase treatment, the partially disintegrated tissue was cultured for 7 days in RPMI 1640 + 20% fetal calf serum to permit cell attachment and outgrowth of endocrine cells. In 17 glands islet-like cell clusters were formed. In stained sections of these newly formed cell clusters frequent mitotic figures were observed. These structures exhibited a high rate of proinsulin biosynthesis and an insulin response to secretory stimuli. Electron micrographs showed a large number of well-preserved granulae-containing cells, including β cells. In the remaining cases lack of growth could be explained by bacterial contamination or loss of viability of the pancreas before dissection. It is concluded that this improved culture technique provides a suitable system for the study of growth and differentiation and perhaps a basis for grafting human fetal endocrine cells to diabetic subjects.

458. Young diabetic patients in the 1930's, 1940's, 1950's and 1960's in Lund, Sweden: how did they manage?

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242 insulin-dependent diabetic patients diagnosed before the age of 22 years in Lund during time periods 1935–1940 (period 1; $n = 56$) 1945–1950 (period 2; $n = 61$), 1955–1960 (period 3; $n = 53$) and 1965–1970 (period 4; $n = 72$) were followed up to January 1983 (periods 1–3) or January 1984 (periods 4). Mortality rate per 1000 person-years due to ketoacidosis for periods 1–4 were 9.9, 2.6, 0.0 and 1.1, respectively. During periods 1–3 deaths due to causes other than ketoacidosis tended to occur after fewer years of diabetes in patients diagnosed between ages 11 and 21 years than in patients diagnosed before age 11 years. The predominant cause of death was renal failure. In general, death occurred earliest in young onset males. There was no time trend during periods 1–3 with regard to deaths due to renal failure for males or females. In period 4 two deaths were found, one due to renal failure and the other to ketoacidosis. At follow-up 25%, 66%, 75% and 97% of these patients were alive from the different periods. The most prominent finding in long-term survivors is the absence of proteinuria.

459. Covalent insulin-receptor complexes are non-cooperative

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B2-nitro, 4-azidophenylacetyl des-Phe^{B1}-insulin inserts covalently and specifically into receptors on irradiation of reversible complexes with ultraviolet light. The receptor pool of IM9-lymphocytes was loaded covalently to various degrees with B2. Cells were incubated for 10 min with normal (1000 ng/ml) and ^{125}I-B2 (10 ng/ml) before irradiation for 2 min. Dissociable insulin was removed by acid washings. The extent of insertion was typically 25–30% expressed as (cpm bound after washing)/(cpm before washing). Control cells, prepared by reversing the incubation/irradiation steps, retained virtually no insulin. Displacement curves were constructed with tracer plus DAA or normal insulin. Scatchard analysis with DAA showed the loss of a fraction of a single receptor class equal to the extent of insertion. Scatchard curves with insulin suggested the loss of a similar fraction of a high-affinity pool, plus the loss of a greater fraction of a low-affinity pool. Covalent loading of the receptor pool had *no* effect on the dissociation rate of reversibly bound tracer at any pH.

460. Multiple insulin injections with the new Novo pen

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A new device in the field of insulin administration – a syringe with the size and appearance of a fountain pen loaded with a prefilled cartridge containing 150 IU of soluble insulin (Actrapid HM, Novo) – was evaluated in clinical practice. Sixteen patients, already on multiple daily injections (mean age 34.9 years, mean duration of diabetes 16 years) participated in a randomised 6 week cross-over study using the new device in one 3 week period (a) and conventional syringes in the other (b). Intermediate-acting insulin was administered once daily using conventional syringes. Metabolic control, as assessed by twice weekly blood glucose profiles, HbA_{1c} and number of hypoglycaemic episodes showed no significant differences between the treatment periods, mean blood glucose values being 10.4 and 10.3 mmol/l, HbA_{1c} being 8.8 and 8.6% in the treatment periods a and b, respectively. Thirteen patients found that the new device made their life easier by simplifying the injection procedure, and 14 patients were still using the device at a 10 month follow-up. In conclusion, the study showed that the new portable insulin syringe was efficient and safe to use. Furthermore, it was preferred by the majority of the patients investigated.

461. Prevalence of urinary tract infection in diabetic women and its relationship to autonomic neuropathy

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Mid-stream urine specimens were collected from 402 consecutive female diabetic patients (15–65 years) attending the clinic. In 47 (12%; 17 with a history of recurrent urinary tract infection (UTI) and 30 asymptomatic) the mid-stream urine showed a viable bacterial count of 10^5/ml or more. Of the 355 women without significant bacteriuria, 81 gave a history of UTI. Thus 128 (32%) had historical or laboratory evidence of past or present UTI. Twenty 'infected' (viable bacterial count 10^5/ml or more) and 21 'uninfected' (viable count $< 10^5$/ml and no previous history of UTI) were randomly selected for cardiovascular autonomic function testing, bladder ultrasound, and mictiography. There were no significant differences between the two groups in age, duration and type of diabetes, HbA_1, blood urea or serum creatinine levels. Abnormalities of cardiovascular autonomic function were significantly greater in the infected group ($p < 0.01$), and they passed a smaller volume of urine on voiding ($p < 0.01$). There were no differences in bladder measurements before and after micturition between the two groups. Urinary infection in female diabetic patients may therefore be related to autonomic neuropathy.

462. In search of the subcutaneous insulin degradation syndrome

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Since the first detailed report of a patient with subcutaneous insulin degradation in 1979, many additional patients have been diagnosed. However, none have met all the criteria of the original syndrome, i.e. (1) normal glucose responsiveness to intravenous, but resistance to subcutaneous insulin; (2) no increase in plasma free insulin following subcutaneous insulin injection; and (3) elevated subcutaneous tissue insulin-degrading activity. Since 1979, we have tested prospectively 14 patients referred for treatment of suspected subcutaneous insulin degradation. Evaluation included multiple insulin challenge tests in an environment preventing patient manipulation. Plamsa glucose and free insulin responses were compared in ten control Type 1 diabetic patients. Alternative explanations were always identified (e.g. factitious disease, communicative disorders, and drug addiction). In addition, we have assayed 20 subcutaneous biopsy specimens for insulin-degrading activity obtained from patients throughout the world with suspected subcutaneous insulin degradation. Insulin degrading activity ranged from 0.9 to 2.5 $fmol \cdot min^{-1} \cdot mg^{-1}$ protein (normal 2.9 ± 1.2 SD). When appropriate hormonal and biochemical criteria are applied, not one case of subcutaneous insulin degradation has been confirmed since 1979. Thus, this syndrome is extremely rare and rigid criteria should be applied if misdiagnosis and inappropriate therapy are to be avoided.

463. Bioactivity and pharmacokinetics of human proinsulin and insulin after intravenous and subcutaneous injections

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The hypoglycaemic actions of biosynthetic human proinsulin and insulin (equimolar ≙ U/kg) were studied after intravenous (IV) and subcutaneous (SC) injection into six rabbits. Immunoreactivity for insulin and human C-peptide (hCP) (before and after binding with protein A-Sepharose-coupled insulin antibodies, 'free hCP') was determined. After insulin IV and SC, blood glucose decreased maximally for 57% and 58% after 20 and 40 min, respectively and recovered again after 3 h. Immunoreactivity for insulin peaked after 5 and 10–30 min, respectively, and decreased according to a process of first order. After proinsulin IV and SC, maximum hypoglycaemia (for 36% and 34%) was reached after 30 and 90 min, euglycaemia after 2 and 4 h, respectively. Immunoreactivity for insulin (and hCP) peaked after 5 and 30–40 min, respectively, and decreased according to a process of higher order. 'Free hCP' was never measurable after proinsulin IV or SC. In conclusion, proinsulin, compared with equimolar amounts of insulin, is potent in vivo. The sustained action of proinsulin SC is

caused by both a delay of resorption and elimination. No evidence was found by specific measurement of free hCP for a contribution of proinsulin conversion to its bioactivity in vivo.

464. Correlations between the rise in plasma non-esterified fatty acids and mean plasma insulin during prolonged exercise in normal, non-diabetic obese and Type-1 (insulin-dependent) diabetic subjects

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We attempted to correlate the change in plasma non-esterified fatty acids (ΔNEFA) during a 3-h treadmill walk (40–50% maximal oxygen consumption) with mean exercise blood glucose and/or plasma insulin (IRI) in three groups of young male subjects tested after an overnight fast under the following conditions: (1) nine lean controls with or without 100 g glucose ingestion; (2) nine obese non-diabetic subjects (126 ± 6 kg) before and after a 13-day protein-supplemented fast (−9 ± 1 kg); and (3) six lean diabetic patients with 100 g glucose ingestion, the overnight intravenous insulin infusion (0.9 U/h) being interrupted or continued during exercise. In controls, glucose ingestion reduced ΔNEFA (398 versus 1070 μmol/l, $2p < 0.001$) without significantly altering blood glucose and plasma IRI. Compared with controls (without glucose), obese subjects showed similar blood glucose, higher IRI (+6.8 mU/l, $2p < 0.005$) and smaller ΔNEFA (−495 μmol/l, $2p < 0.02$); the protein diet lowered blood glucose and IRI (−5.7 mU/l, $2p < 0.001$) and enhanced ΔNEFA (+300 μmol/l, $2p < 0.05$). Compared with controls (with glucose), insulin-infused diabetic patients showed higher blood glucose (+4.1 mmol/l, $2p < 0.001$) and smaller ΔNEFA (−239 μmol/l, NS); insulin deprivation was associated with an additional rise in blood glucose (+8.4 mmol/l, $2p < 0.001$) but no significantly greater ΔNEFA (+143 μmol/l). Thus, exercise-induced ΔNEFA is negatively correlated with IRI ($r = -0.67$; $n = 26$ in normal and obese subjects; $p < 0.001$) and is also inhibited by glucose administration, even during acute insulinopenia.

465. Effect of growth hormone treatment on insulin sensitivity and insulin receptor binding in paients with growth hormone deficiency

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Disturbances of carbohydrate metabolism are well known in patients with growth hormone (GH) deficiency. However, the effect of GH treatment on insulin sensitivity in this disorder has not as yet been studied. Thus, we investigated the influence of GH on insulin sensitivity and insulin receptor binding by means of the euglycaemic clamp technique and radioreceptor assay using mononuclear leucocytes as target cells. Five patients with hypopituitarism were studied during GH therapy (phase A) and after withdrawal of treatment for 4 weeks (phase B). Informed consent from the patients and their parents for participation in the study was obtained. Results: (1) basal insulin levels were not different in phases A and B. (2) Peripheral glucose utilisation was significantly elevated in phase B compared with phase A (6.3 versus 3.9 mg/kg body weight per min, $p < 0.05$). (3) Insulin receptor binding to monocytes remained unchanged after withdrawal of GH therapy (specific binding fraction 3.18% versus 3.66%, NS). In conclusion, these data indicate a significant influence of GH substitution on peripheral glucose metabolism in patients with GH deficiency, which is probably due to a post-receptor mechanism.

466. Non-enzymatic glycosylation of LDL does not alter its recognition by the high affinity receptor

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Serum low density lipoproteins (LDL) of diabetic subjects are non-enzymatically glycosylated to a higher extent compared with normal subjects. The possible effect of this post-ribosomal modification on LDL-catabolism was investigated. LDL was isolated by sequential ultracentrifugation, iodinated with ^{125}I, and glycosylated by incubation with glucose (55–1100 mmol/l) for 3 days in the presence and absence of $Na(CN)BH_3$. The extent of glycosylation was estimated by determination of furosine and by titration of unreacted lysine residues. Internalisation and degradation of LDL species at low and physiologically high concentrations (5–2200 μg cholesterol/ml) were studied with cultured human fibroblasts. No effect of glycosylation on LDL catabolism was found. However, catabolism of reductively glycosylated LDL (23% of lysines modified) was reduced by 90%. Comparing non-reductively and reductively glycosylated LDL slower degradation occurred with the latter even when the same amount of lysine residues was modified. Obviously it is the chemical nature of the modification rather than the number of lysine residues that is recognized by the high affinity receptor indicating that reductive glycosylation is not a useful tool to study the possible effect of hyperglycaemia on LDL catabolism.

467. Morphometrical investigations of diabetic microangiography and autonomic neuropathy of the gut

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To date there has been no electron microscopic study of vascular and neural lesions of the human gut in diabetes mellitus, and light microscopical investigations are inconclusive. Methods: rectal biopsies from 20 healthy controls (group 1), 20 diabetic patients without gastrointestinal symptoms (group 2), and from 7 diabetics with severe gastrointestinal motility disorders (group 3) were studied by electron microscopy. Morphometry of the capillary basal laminar thickness (10 capillaries per patient) employing the method of Siperstein, and morphometry of the area of each cross-sectioned axon (on average 142 axons per patient) were performed with a manual optic processing system. Results: in groups 2 and 3 the capillary basement membranes showed identical, significant thickening ($p < 0.01$) compared with group 1. The axons in group 3 revealed significant swelling concomitant with a reduction in intra-axonal organelles compared with group 1 ($p < 0.05$). In group 3 most patients showed an intra-neural increase of lysosomes and lipofuscin, and four out of seven patients had multilayered thickening of the basal lamina of the Schwann cells. Conclusions: patients with long-standing diabetes can develop gastrointestinal microangiopathy. In most patients with diabetic motility disorders an intrinsic autonomic neuropathy of the gut is revealed by electron microscopy.

468. Glucagon-like peptide 1 but not glucagon-like peptide 2 augments glucose-induced insulin release in isolated rat pancreatic islets

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Glucagon-like peptide 1 (GLP-1) and glucagon-like peptide 2 (GLP-2) are arranged in tandem on the m-RNA of pancreatic pre-proglucagon. The GLP-1 sequence is identical in the human, bovine and hamster glucagon precursor, whereas minor differences exist between the corresponding GLP-2 sequences indicating substantial conservation in evolution. We investigated the insulin-releasing activity of chemically synthesized GLP-1 and GLP-2 in collagenase isolated rat pancreatic islets kept in short-term tissue culture. Islets were incubated in Krebs-Ringer bicarbonate buffer for 30 min in the presence of glucose at 2.8 mmol/l or 16.7 mmol/l. GLP-1 and GLP-2 were added up to a concentration of 0.1, 1 or 10 μg/ml. Results: the insulin release at 2.8 mmol/l glucose was 104 ± 9 mU/l per 30 min ($n = 19$) and at 16.7 mmol/l 213 ± 10 mU/l per 30 min ($n = 24$). GLP-1 stimulated insulin release at 16.7 mmol/l glucose in a dose-dependent manner: at 0.1 μg/ml the insulin release reached 263 ± 27 mU/l per 30 min ($n = 11$, $p < 0.05$), at 1 μg/ml 314 ± 24 mU/l per 30 min ($n = 20$, $p < 0.001$) and at 10 μg/ml 339 ± 28 mU/l per 30 min ($n = 6$, $p < 0.001$). Under identical conditions, GLP-2 did not stimulate insulin release. GLP-1 augments glucose induced insulin release from rat pancreatic islets, whereas GLP-2 does not show this activity.

469. Obesity and mortality in maturity-onset diabetes

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The influence of obesity on mortality in Type 2 (non-insulin-dependent) diabetes was studied. 609 patients (aged 50–75 years, diagnosed after the age of 45 years) were observed over 10 years (1973–1983). They were grouped according to duration of diabetes and urinary albumin excretion. Since an increase of the latter is known to be an important risk factor, we concentrated on patients with low urinary albumin excretion (< 15 μg/ml in morning urine samples). The material was subdivided according to weight in percentage of ideal body weight. For these sub-groups, the average blood pressure, fasting plasma glucose level, and modality of treatment was registered as well. Except for a slight difference in diastolic blood pressure, the groups were comparable. The preliminary analyses were performed on 165 patients with duration of diabetes of < 5 years, low urinary albumin, and mean observation time 9.5 years. The following increases in mortality in comparison with the background population were ob-

served: percentage ideal body weight < 90 ($n = 14$) 40%; > 90 ≤ 110 ($n = 81$) 19%; > 110 ≤ 130 ($n = 50$) 26%; > 130 ($n = 20$) 148%, the latter increase only was statistically significant ($2p = 0.001$). These results indicate that increased mortality in elderly primarily Type 2 diabetic patients is not related to obesity unless severe (i.e. > 130%).

470. Severe insulin resistance in Type 1 (insulin-dependent) diabetic patients with ureamia assessed by the euglycaemic clamp technique

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This study was designed to compare insulin resistance in Type 1 diabetic patients with normal kidney function (IDD), uraemic Type diabetic patients (UIDD) and non-diabetic uraemic patients (U). A three-step euglycaemic clamp was performed in nine healthy subjects (C), 10 IDD, 10 U, five non-dialysed UIDD and 10 dialysed UIDD. Three rates of insulin was infused, each during 120 min: 0.5, 2.0 and 4.0 $mU \cdot kg^{-1} \cdot min^{-1}$. As expected the maximal glucose uptake was lower in IDD and U than in C (13.8 ± 0.7 and 10.2 ± 0.7 versus 16.2 ± 1.0 $mg \cdot kg^{-1} \cdot min^{-1}$; $p < 0.01$). However, the maximal glucose uptake of the non-dialysed UIDD was still lower (8.4 ± 0.9 $mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.05$) compared with U and IDD. In the chronic dialysed UIDD, maximal insulin mediated glucose uptake was significantly improved (11.8 ± 0.6 $mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.01$), especially in five subjects on continuous ambulatory peritoneal dialysis in whom insulin was administered intraperitoneally (13.2 ± 0.5 $mg \cdot kg^{-1} \cdot min^{-1}$). Serum free insulin and blood glucose were similar in all groups. The insulin levels required to elicit half-maximal response were higher in IDD, U and UIDD (81 ± 14, 68 ± 6 and 67 ± 13 mU/l) than in C (40 ± 8 mU/l; $p < 0.03$). In conclusion, uraemic Type 1 diabetic patients exhibit severe and superimposed insulin resistance. The mechanism is probably caused by depressed insulin action at various post-binding steps.

471. Counter-regulatory hormones during hypoglycaemia

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To determine the hazards of continuous subcutaneous insulin infusion, we investigated eight C-peptide-negative diabetic patients. After simulating a pump failure they developed mild ketosis with a mean blood glucose of 20.7 ± 1.2 mmol/l, pH 7.38 ± 0.01, non-esterified fatty acids 1.14 ± 0.24, acetoacetate 0.733 ± 0.109 and hydroxybutyrate 1.725 ± 0.355 mmol/l (mean ± SEM). Blood glucose then was lowered by 25%/h with glucose-controlled insulin infusion at 2.7 ± 0.1 mmol/l (insulin requirement 37 ± 6 IU, time 367 ± 25 min). All patients complained of symptoms of hypoglycaemia. At blood glucose nadir, growth hormone ($+912 \pm 347$ pmol/l) and ACTH ($+14 \pm 5$ pmol/l) rose significantly ($2p = 0.05$), while adrenaline ($+250 \pm 126$ pg/ml, $2p = 0.1$) did not. In addition, cortisol and noradrenaline showed no increase. Glucagon (-18 ± 7 pmol/l and Dopamine (-90 ± 31 pg/ml) were significantly suppressed by insulin. In the individual patient at least two and at most five of the above mentioned hormones were elevated, but the pattern was not predictable. We found a positive correlation between the age at manifestation of diabetes and the ACTH increment ($r = +0.75$) and a negative correlation between insulin dose/day and ACTH increment ($r = -0.80$). Thus, not a single but only a combination of hormones substantiated hypoglycaemic counter-regulation.

472. Effect of α-amylase inhibitor (Tendamistat) on post-prandial blood glucose, serum insulin and C-peptide levels in Type 2 (non-insulin-dependent) diabetic patients

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Modulation of absorption processes induced by specific inhibitors retarding the breakdown of carbohydrates is considered as an adjunct to sulphonylurea treatment. Recently, a polypeptide (Tendamistat); inactivating salivary and pancreatic α-amylase irreversibly, has been developed. Thus, we studied the effect of Tendamistat (3×200 mg daily) in 15 obese Type 2 diabetic patients with poor metabolic control during sulphonylurea treatment. All patients, receiving a standardized diet (3 mixed meals/day consisting of 35, 52 and 34 g carbohydrates), were treated for 3 days with either Tendamistat or placebo in a double-blind cross-over study and were tested for blood glucose, insulin and C-peptide diurnal profiles. The maximal blood glucose increase after meals was significantly lower during Tendamistat treatment compared with placebo: after breakfast: 2.3 ± 0.6 versus 5.3 ± 1.4 mmol/l, $p < 0.0005$); after lunch: 0.9 ± 0.2 versus 2.7 ± 0.7, $p < 0.0005$). Consequently, the diurnal blood glucose profiles were significantly lower during Tendamistat treatment compared with placebo ($p < 0.0005$ at 9.00, 11.00, 13.00, 15.00 h). Meal-stimulated plasma insulin and C-peptide values were significantly lower with Tendamistat. The present data indicate that post-prandial hyperglycaemia can be smoothed by the α-amylase inhibitor (Tendamistat) in Type 2 diabetic patients with insufficient metabolic control during sulphonylurea treatment. The significantly lowered blood glucose diurnal profiles during treatment were associated with decreased meal-stimulated insulin and C-peptide values.

473. Immunocytochemical demonstration of insulin in pancreatic islet lysosomes as evidence of a crinophagic mechanism for intracellular insulin degradation

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The aim of the present investigation was to study further the role of lysosomes in intracellular insulin degradation of isolated pancreatic islets. To achieve a metabolic steady state, islets were kept in tissue culture for one week, at either 3.3, 5.5 or 28 mmol/l glucose. They were then prepared for electron microscopy. To demonstrate insulin specifically within the islet lysosomes, immunocytochemical staining, using the immunogold method, was employed. Primary and secondary lysosomes were identified on the basis of morphological and enzyme histochemical criteria. Within the group of secondary lysosomes, a subgroup, consisting of immunocytochemically stained lysosomes, was recognized. At each one of the glucose concentrations, the lysosomal populations were evaluated by morphometric methods. It was clearly shown that material resembling secretory granules, located within secondary lysosomes, was composed of insulin. The percentage of insulin-containing secondary lysosomes was highest at 5.5 mmol/l, lower at 28 mmol/l and lowest at 3.3 mmol/l glucose. In conclusion, the present data show that crinophagy in pancreatic islets provides a mechanism for intracellular insulin degradation. The results also suggest that the crinophagic process is probably determined by the actual amount of granule-bound insulin in the islet β cells.

474. A 3 month, placebo-controlled, efficacy study of sorbinil for diabetic neuropathy

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An increased polyol-pathway activity probably plays a role in the genesis of diabetic neuropathy. We studied the effect of sorbinil – a potent inhibitor of the key polyol-pathway enzyme aldose reductase – on diabetic polyneuropathy and autonomic neuropathy in a randomized double-blind parallel group placebo controlled study. A 4-week placebo run-in was followed by a 12-week period with sorbinil (200 mg daily) or placebo, followed by a 4-week washout. Of 32 recruited patients with diabetic neuropathy, 28 completed the study. Efficacy variables were assessed at screening and at 4-week intervals thereafter. When the results before and after sorbinil treatment were compared with the results before and after placebo, three of the ten measured nerve conduction variables improved significantly: median motor nerve conduction (m/s): 54.94 ± 1.38 to 56.95 ± 1.54 (sorbinil) versus 57.15 ± 1.53 to 55.38 ± 1.93 (placebo) ($p < 0.05$), peroneal distal latency (ms): 4.66 ± 0.31 to 4.27 ± 0.32 (sorbinil) versus 4.63 ± 0.35 to 4.55 ± 0.28 (placebo) ($p < 0.02$) and Hoffman H-M interval (ms): 33.81 ± 1.12 to 32.75 ± 1.00 (sorbinil) versus 33.40 ± 0.91 to 33.04 ± 0.89 (placebo) ($p < 0.05$). Glycaemic control was constant during the study. No clear evidence of effect of sorbinil was obtained on: pain scores, vibratory perception threshold and cardiovascular reflex test. It is concluded that sorbinil may improve some nerve conduction values. However, the clinical applicability of these observations remains to be determined.

475. Relationships between the cardiovascular and gastrointestinal systems: biphasic glucose response to a meal – a typical sign of gastric involvement

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In 40 diabetic patients autonomic neuropathy (AN) of the cardiovascular system was investigated and compared with data on gastric emptying. The following methods were used (1) for the study of cardiovascular involvement: (a) heart-rate variation during deep breathing, (b) during Valsalva-manoeuvre, (c) Schellong-test; (2) for gastric involvement: (a) functional scintigraphy with a ^{99m}Tc-DTPA-

labelled semi-fluid test meal and (b) euglycaemic clamp. Twenty patients showing one or less abnormal cardiovascular testresults were defined as a lower-grade group, the 20 others having? ⩾ two pathological tests as a higher-grade group. Functional scintigraphy revealed a decrease of activity to ⩽60% after 30 min in 4/20 higher-grade compared with 0/20 lower-grade patients and to <45% after 60 min in 10/20 compared with 6/20. This indicates accelerated gastric emptying of the semi-liquid test meal in the higher-grade group. Clamp studies performed during ⩾ three normal, solid-liquid meals, indicated a delayed gastric emptying of these foods. The time-difference between increase and maximum blood glucose was >35 min in 9/20 higher-grade and only 2/20 lower-grade patients. Interestingly 7/20 higher-grade compared with 1/20 lower-grade patients showed a biphasic curve during clamping. This seems to be a typical sign of AN and may result from accelerated gastric emptying of liquid and delayed emptying of solid foods.

476. Conditions of glucose-increased cyclic AMP levels in the pancreatic βcell

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Studies on purified islet cells have attributed the poor glucose-induced insulin release from single βcells, in part, to a deficiency in cellular cyclic AMP levels. These results question the capability of glucose to induce cyclic AMP formation in pancreatic βcells. In the present study, the effect of glucose upon cyclic AMP production was measured in purified βcells, either in the presence or absence of other islet cell types or their secretory products. Increasing the glucose concentration from 1.4 to 20 mmol/l did not elevate cyclic AMP levels in single or reaggregated βcells; nor was this the case in purified non-βcells. During glucagon-induced cyclic AMP formation, 20 mmol/l glucose provoked a 30% rise in cyclic AMP content of purified βcells. This glucose effect increased in the presence of somatostatin, which inhibited glucagon-induced cyclic AMP formation more markedly at 1.4 mmol/l than at 20 mmol/l glucose. Glucose also elevated cyclic AMP levels in intact islets and in newly formed aggregates of islet β and non-βcells incubated without exogenous pancreatic hormones. It is concluded that glucose alone does not induce cyclic AMP formation in pancreatic βcells, but rather amplifies cyclic AMP production generated by locally released or circulating effectors.

477. A computerized program for intensified subcutaneous insulin therapy by diabetes self-adjustment

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Generally, it is accepted that blood glucose self-monitoring is essential to the management of diabetes mellitus. However, often it is very difficult for patients and physicians to prescribe the correct insulin dose from measured blood glucose. Therefore, we have developed a computerized program for the adjustment of daily insulin doses. Its essential part is a control system which compares real and nominal blood glucose values and which then calculates the new insulin dose in consideration of the blood glucose values and insulin doses of the past 3 days. Four blood glucose measurements (pre-prandial, before lunch, dinner, and night) are necessary. Minimum/maximum insulin doses are fixed individually. In 20 insulin-dependent diabetic in-patients treated in this way blood glucose levels dropped from 16.7 mmol/l at admission to nearly normoglycaemia (post-prandial 8·3 mmol/l, mean blood glucose level 6·1 mmol/l and stabilized after 1 week (MAGE) 2·78 mmol/l). Severe hypoglycaemic episodes were reduced. After first stabilization the daily insulin doses fluctuated ± 2 to 4 IU. Computerized insulin adjustment may be a valid instrument for diabetes stabilization. A low-priced version of our program using a pocket-computer is in preparation for out-patient treatment.

478. Decreased forearm uptake of glucose during amino-acid infusion

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Increased plasma glucose concentrations resulting from decreased glucose utilization have been observed during leucine infusion in prolonged fasting in man. To determine whether leucine decreases glucose flux ($^2H_2$-glucose) and forearm glucose uptake in man, normal overnight-fasted subjects were infused with saline ($n=6$) or leucine (0.5 or 1.0 μmol·kg^{-1}·min^{-1}, $n=10$) for 4 h. Basal forearm glucose uptake was similar in all three groups. Over the course of the study, plasma glucose concentrations and glucose flux decreased ($p<0.05$), but no differences among groups were observed. Following saline infusion, forearm glucose uptake was not changed ($\Delta = +6.1 \pm 13.3$ μmol·min^{-1}·dl^{-1} forearm volume) while leucine infusion decreased ($p<0.05$) forearm glucose uptake ($\Delta = -26.7 \pm 12.8$, -21.7 ± 6.7 μmol·min^{-1}·dl^{-1} forearm volume for low and high leucine doses, respectively). Plasma insulin and C-peptide concentrations decreased during infusions of saline and the lower leucine dose, but were unchanged during the higher dose. A correlation ($r=-0.79$, $p<0.001$) was found between the change in glucose uptake and change in leucine carbon uptake. In summary, increased leucine uptake is associated with decreased glucose uptake in the forearm of man. In conclusion, amino-acids may supplant glucose as an oxidative fuel in forearm tissue.

479. Insulin ouptut, ion flux and electrical measurements show that mouse islets distinguish fast from slow changes of glucose concentration

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The response of perifused isolated mouse islets of Langerhans to a glucose challenge depends on the rate at which the glucose concentration changes. With steep gradients (31 mmol·l^{-1}·min^{-1}), resulting from rapid flow rates (1.0 ml/min), perifused collagenase-treated islets responded by raising their insulin output to twice basal level within 5 min reaching a maximum in 10 min. At lower flow rates, for example 0.3 ml/min (13.8 mol·l^{-1}·min^{-1}), the insulin output took 30 min to double and took more than 40 min to reach a maximum. In contrast, lactate output measured simultaneously followed the glucose concentration. In parallel experiments rubidium fluxes showed that the potassium permeability decreased rapidly by approximately 50% but with shallow gradients this change was reduced. Simultaneous insulin and electrical measurements on single microdissected islets are consistent with the above results. They show that changing the glucose concentration from 1.2 to 22.2 mmol/l at 31 mmol·l^{-1}·min^{-1} produces a typical biphasic electrical response, whereas the electrical activity and insulin output following the same change made at 2.1 mmol·l^{-1}·min^{-1} produces a significantly reduced response.

480. Regulatory effects of insulin on cholesterol metabolism

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To clarify the role of insulin in changes in the activities of the rate-limiting enzymes of cholesterol metabolism in diabetic rats, we examined the cholesterol enzymes in vagotomised animals, since the vagus nerve controls insulin release. Sham-operated and vagotomised normoinsulinaemic rats, hypoinsulinaemic diabetic rats and hyperinsulinaemic obese rats were investigated. Vagotomised diabetic rats had increased HMG CoA reductase (0.024 ± 0.002 versus 0.013 ± 0.051 nmol·min^{-1}·mg^{-1} $p<0.001$), decreased ACAT (0.119 ± 0.003 versus 0.174 ± 0.002 $p<0.001$) and decreased 7α hydroxylase activity (25.5 ± 2.5 versus 55.1 ± 5.4 $p<0.001$) in comparison with control rats. Vagotomised obese rats had increased HMG CoA reductase (0.472 ± 0.048 versus 0.182 ± 0.015 $p<0.001$), similar ACAT activity, decreased 7α hydroxylase activity (27.15 ± 1.68 versus 42.46 ± 3.06 $p<0.001$). This increase in the rate of cholesterol synthesis together with its decreased utilisation led to increased levels of cholesterol in the tissues and serum. These results suggest a major role for insulin in regulating cholesterol metabolism. Thus diabetic rats with hyperinsulinaemia or autonomic neuropathy may have an even greater risk of developing atherosclerotic complications.

481. Long-term development of diabetic glomerulopathy in normal and neonatally uninephrectomized rats

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Development of renal glomerular structural alterations has been studied at regular intervals from 5 weeks to 1.5 year in neonatally uninephrectomized rats with streptozotocin diabetes (blood glucose 21.1 ± 3.4 mmol/l; mean ± SD). (All differences stated below are statistically significant unless otherwise specified). The total glomerular volume in the diabetic rats was increased by 33%. Uninephrectomy increased the total glomerular volume by 81% in non-diabetic rats and by a further 12% (NS) in the diabetic rats. The whole kidney weight was increased by 39% in the diabetic rats, by 84% in non-diabetic uninephrectomized rats and by a further 28% in the diabetic rats. With the exception of the total glomerular volume in control rats, neither kidney weight nor glomerular volume changed with age in any group. The tail blood pressure was unchanged from control value (121 ± 8 mmHg) by diabetes, uninephrectomy or their combination. The

heart rate was decreased by 43 ± 14 beats/min, (mean ± SEM) in the diabetic rats without relation to uninephrectomy. In conclusion, long-term experimental diabetes alone or in combination with uninephrectomy, does not produce hypertension, but induces marked changes in kidney and glomerular size.

482. A multicentre epidemiological study on the prevalence of diabetic retinopathy

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An epidemiological study on the prevalence of diabetic retinopathy in a diabetic population living in the North East of Italy (Veneto region) was performed. A protocol, adapted to computer analysis, was prepared for collection of data. The 12 diabetic clinics involved in the study were located in different geographical areas (mountain, plainland, seaside) to evaluate a possible influence of climatic and behavioural factors. The whole diabetic population was represented by 42,975 subjects who were divided at every clinic into four groups, according to the duration of diabetes (< 5, 5–10, 10–20, > 20 years). A preliminary statistical analysis showed that 1,429 subjects were sufficient to obtain the real prevalence with a deviation of ± 2.7% in our population; therefore we have conducted our study on 1,429 randomly selected diabetic patients. The results obtained on 1,114 subjects (10 out of 12 centres) are reported. General prevalence of diabetic retinopathy was 31% (proliferative 3%, non-proliferative 97%). The following data were also collected: prevalence in the duration of diabetes; frequency of maculopathy; relationship between retinopathy, and: familiarity, duration, type and therapy of diabetes, metabolic control (glycaemia, HbA_{1c}), renal function (proteinuria), hypertension, smoking and alcohol intake.

483. Insulin therapy in overweight Type 2 (non-insulin dependent) diabetic patients: an association with higher prevalence of retinopathy

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Insulin treatment in overweight Type 2 diabetic patients has been associated with increased insulin resistance, which is a further pathogenetic factor of retinopathy. To establish whether previous insulin treatment is related to raised prevalence of retinopathy in over-weight subjects with Type 2 diabetes, we studied 23 normotensive outpatients (11 males, 12 females), who were admitted to our clinic with body mass index ⩾ 25 kg ÷ m^2, length of illness ⩾ 10 years and who had been treated for at least 3 years with insulin ($n = 14$) or sulphonylureas ($n = 9$). Diagnosis of retinopathy was performed by fluorescein angiography. Insulin-treated patients did not differ from diabetic subjects on oral drugs (mean ± SD, fasting basal C-peptide levels 0.65 ± 0.2 versus 0.76 ± 0.3 nmol/l; fasting blood glucose 11.4 ± 2.3 versus 10.5 ± 2.8 mmol/l; HbA_1 11.5 ± 2.3 versus 11.6 ± 2%; age 57 ± 8 versus 58 ± 7 years; length of illness 19 ± 6 versus 16 ± 7 years and median body mass index 27.7 versus 27.4 kg ÷ m^2). Prevalence of retinopathy was significantly higher in the group on insulin (13 out of 14; 10 background, 3 proliferative) than on sulphonylureas (1 out of 9; background); $\chi^2 = 12.13$ (Yates correction), $p < 0.005$. Our data suggest that previous insulin therapy in Type 2 overweight diabetic patients is associated with a higher prevalence of retinopathy.

484. Increased βcell pH stimulates insulin release

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Elevated glucose concentrations increase the intracellular pH in mouse β cells (pH_i). This raises the question whether alkalinization of the β cells, as such, can be a step in the triggering of insulin release. We have tested whether three situations, known to increase pH_i in other cells, also induce insulin release in mouse islets maintained in Hepes-buffered medium without HCO_3/CO_2 and with 1 mmol/l D-glucose. Parallel measurements were performed of insulin release in perifusion and in static 60-min incubations and of pH_i, measured as the uptake of ^{14}C-5,5-dimethyloxazolidine-2,4-dione (DMO). HN_4^+ (5 mmol/l) acutely increased pH_i and reversibly induced insulin release. Stepwise change of extracellular pH (pH_o) from 6.8 to 8.0 resulted in parallel but smaller changes in pH_i. Insulin release (60 min) was not affected at pH_o 6.8–7.6 but increased at 7.8–8.0. Acute changes from pH_o 7.4 to 8.0 promptly induced reversible insulin release. Change from HCO_3/CO_2-containing medium to one lacking this buffer increased pH_i and promptly induced insulin secretion. These results strongly suggest that alkalinization of the β cell can activate the insulin secretory system.

485. Beneficial effect of effective insulin therapy with pumps on non-ischaemic forms of diabetic retinopathy

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Recent studies have suggested that near-normalization of blood glucose with insulin pumps cannot prevent the development of proliferative retinopathy in patients with background retinopathy. We have studied 12 Type 1 (insulin-dependent) diabetic patients treated permanently by intraperitoneal insulin infusion for 23 ± 4 months. All the subjects had background retinopathy *without* ischaemic areas on fluorescein angiograms and had not received photocoagulation. Patients were allocated to two groups according to the degree of glucose control (HbA_1 above or below 8.5%, normal range 5.5–8.5%), although glycaemic control was significantly improved in all cases when compared with previous conventional therapy. Angiography changes were interpreted quantitatively (−2 to +2, arbitrary units) and 'blindly' by three independant ophthalmologists. The results showed seven improvements, four stabilizations and one aggravation, although not progressing to proliferative stages. Patients with good control ($n = 6$, HbA_1: 7.7 ± 0.4%) showed a better angiogram score than those with average control ($n = 6$, HbA_1 9.8 ± 0.5%): + 1.0 ± 0.2 versus + 0.2 ± 0.3 respectively ($p < 0.05$). These results suggest that further controlled randomized studies limited to non-ischaemic background retinopathy are needed to re-assess the influence of near-normalization of blood glucose on retinal morphology.

486. The influence of insulin therapy on myelinated nerve fibre maturation and body growth in streptozotocin-diabetic rats

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Three groups of Sprague-Dawley rats were studied over 2 months: controls, untreated diabetic and diabetic animals treated with daily injections of long-acting insulin. Satisfactory control of blood glucose levels was achieved. Body weight increased in the treated animals in parallel with the controls but declined in the untreated diabetic rats. Tibial length increased in the control and treated animals ($p < 0.001$) but was unchanged in the untreated diabetic animals. Total myelinated fibre number in tibial nerves decreased in both treated and untreated diabetic animals when compared with non-diabetic controls ($p < 0.01$). Fibre diameter increased between onset and end controls ($p < 0.001$). There was no significant difference between the value for the untreated diabetic animals and that for onset controls, but it was significantly less when compared with the end controls ($p < 0.001$). For the treated diabetic rats mean fibre diameter was less than that of the end controls ($p < 0.001$) and was slightly less than the untreated diabetic and onset control animals but this did not reach statistical significance. Thus, insulin treatment was found to normalize body weight, improve skeletal length but nerve fibre number and diameter were uninfluenced.

487. Unexpected potentiation of glucose-stimulated insulin release by calcium store blockers

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The muscle relaxant, Dantrolene, and TMB-8 (8[8-(N,N-Diethylamino)octyl 3,4,5-trimethoxybenzoate) are Ca^{++} antagonists which block Ca^{++} efflux from intracellular stores without affecting Ca^{++} flux into the stores. Under low glucose conditions the antagonists are without effect on insulin release. In accord with an action of blocking efflux of stored calcium, $^{45}Ca^{++}$ efflux from pre-loaded islets was markedly decreased by administration of calcium store antagonists. Furthermore, islets incubated with Dantrolene for 30 min exhibited, on removal of the drug, increased $^{45}Ca^{++}$ efflux and a concomitant increase in insulin release. This is assumed to be due to the store accumulating calcium, as efflux is blocked with influx unchanged, followed by unloading of Ca^{++} into the cytosol as the drug is washed out. Surprisingly, the Ca^{++} store blockers potentiated stimulated insulin release, e.g., when sub-maximal, but not maximal, glucose concentrations were used. The results are explained by an action of the drugs on raising the set point at which the stores regulate intracellular Ca^{++} as the stores fill. The data suggest that the filling of an intracel-

lular store by Ca^{++} and subsequently raised set point is involved in the mechanism underlying the second phase of insulin release.

488. Appearance of insulin-receptor antibodies in mice and man

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Mice immunized to insulin develop anti-insulin (idiotype) and subsequently develop anti-anti insulin (anti-idiotype). The anti-idiotype interacts with the insulin receptor, and can mimic all the biological actions of insulin in vitro. Mice developing anti-idiotypic receptor antibodies have disturbances in their glucose homeostasis. The physiological disturbances are associated with 'down-regulation' and 'desensitization' of insulin receptors. Studies in human patients have indicated that certain juvenile diabetic patients had insulin receptor antibodies of the IgM class in their sera. These antibodies appeared in the circulation before the patients had received insulin therapy. A child suffering from severe hypoglycaemic attacks also has receptor antibodies of the IgM class in its circulation. In this case the antibodies seem to be directly responsible for the severe hypoglycaemic attacks, since medical immunosuppressive treatment resulted in the synchronous disappearance of the receptor antibodies and the hypoglycaemic attacks.

489. Four years of experience with glycosylated haemoglobin measurements in a diabetic clinic

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Glycosylated haemoglobin (HbA_1) measurement has been performed routinely on all diabetic out-patients since 1979. Since 1981, the diabetic clinic has been re-organized so that the results have been available at the time the patient is seen. Analysis of the data so far allows the following conclusions: (1) the frequency distribution of HbA_1 measurements over this period ($n = 5445$) shows a range of 4–26.7% (mean $10.9 \pm 2.5\%$); (2) simultaneous clinic (post-prandial) capillary blood glucose and HbA_1 measurements do correlate ($n = 5445$, $r = 0.52$, $p < 0.0001$), the correlation being greater for patients receiving oral hypoglycaemic therapy ($n = 718$, $r = 0.62$, $p < 0.0001$); (3) mean HbA_1 values do not differ significantly between the sexes; (4) in 255 patients selected at random, a significant correlation has been found between HbA_1 measurements and duration of diabetes ($r = 0.33$, $p < 0.001$); (5) the mean HbA_1 has shown a significant fall over the period studied from 11.5% in the last quarter of 1979 to 10.6% for the first 8 months of 1983 ($p < 0.001$). In our clinic, the immediate availability of HbA_1 measurement has been demonstrated to lead directly to a change in management in many patients. The significant fall in HbA_1 values over the period concerned suggests this to be beneficial to diabetic control.

490. Are general practioners and nurses suitable to educate diabetic patients?

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In Hungary the vast majority of diabetic patients are managed by general practitioners (GP) and qualified nurses. Education of diabetic patients is considered to be a task for them. We carried out a questionnaire survey to judge the level of diabetes-related knowledge of 60 GP, 24 ward-doctors (in internal medicine), 53 nurses and 51 diabetic patients tested before and after a diabetes teaching course, respectively. The results of different groups (expressed in percentage of possible maximum score) were: 78% for ward-doctors, 69% for patients after education, 63% for GP, 43% for patients before education and nurses, respectively. The distribution of right and wrong answers in different groups was compared and analysed by χ^2 test. We conclude that (1): the knowledge of diabetes can be raised significantly by education ($p < 0.001$). (2) The knowledge of GPs is more than that of non-educated patients, but less than that of educated diabetic subjects ($p < 0.001$); (3) the education level of ward-doctors is greater than that of GPs ($p < 0.001$); (4) The knowledge of nurses and non-educated patients is similar ($p > 0.1$).

491. Metabolic changes in men developing diabetes during one decade

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During 1970–1973 all men living in Uppsala and born 1920–1924 were invited to participate in a health survey. Of the 2322 who participated, 1801 were restudied in 1980–1983 to evaluate the incidence of Type 2 (non-insulin dependent) diabetes. Criteria for the diagnosis of Type 2 diabetes at re-investigation were (A) treatment with sulphonylurea or biguanide drugs or both, (B) fasting-blood glucose $\geq$ 6.7 mmol/l on *two* occasions, (C) fasting blood glucose ≥ 6.7 mmol/l *and* blood glucose 120 min after an oral glucose load (75 g) $\geq$ 10.0 mmol/l, or (D) fasting blood glucose ≥ 6.7 mmol/l *and* k-value at an intravenous glucose tolerance test (0.5 g/kg body weight – k_{IVGTT}) of ≤ 0.9. Sixty-two (3.3%) of men normoglycaemic in 1970–1973 were found to have developed Type 2 diabetes when retested. A preliminary analysis shows that k_{IVGTT} in 1970–1973 and 1980–1983 was 1.18 and 0.72 respectively ($p < 0.001$), fasting serum insulin 20.8 and 12.5 mU/l respectively ($p < 0.001$), the insulin index (ratio between mean of 4, 6 and 8-min insulin values and fasting serum insulin) 2.79 and 1.20 respectively ($p < 0.05$). Body weight was on average unchanged over the time period. Fifty-year-old men, who in the next decade developed Type 2 diabetes, decreased their fasting serum insulin but still more their peak-insulin value and insulin index.

492. Evidence for insulin-specific T-cell immunity in a patient with hypoglycaemia and insulin autoantibodies

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A 55 year old lady presented with spontaneous hypoglycaemia associated with elevated fasting plasma insulin (105 mU/l) and proinsulin (480 pmol/l) but normal C-peptide. Plasma free insulin (PEG extraction) was repeatedly normal, suggesting insulin autoantibodies. ^{125}T-insulin binding of serum was modestly increased, and anti-insulin IgG and IgM were not detectable (Novo Research Institute), suggesting high affinity autoantibodies. Insulin clearance was fivefold lower than in normal subjects, and insulin sensitivity of glucose metabolism was normal. Proliferation of blood lymphocytes caused by insulin was assessed in the patient, in six healthy control subjects and in 24 Type 1 (insulin-dependent) diabetic patients. Proliferation rates were significantly elevated in the patients. since stimulation index (cpm insulin/cpm medium) for human, pork and beef zinc insulin was 70 ± 23, 59 ± 21, 137 ± 24 compared to 12 ± 2, 13 ± 3, 19 ± 4 in control subjects and to 9 ± 2, 11 ± 2, 15 ± 3 in diabetic patient. The proliferating lymphocytes in the patient were T-cells as demonstrated by T-cell markers in long-term cultures, responding upon restimulation with insulin and autologous adherent cells. T-cell phenotype was 65–85% T4 (helpers/inducers) and 10–14% T8 (suppressors/cytotoxic cells). The findings provide evidence for autoimmunity caused by aberrant T-cell clones, they demonstrate that spontaneous hypoglycaemia with insulin autoantibodies is associated with circulating T-cells abnormally responsive to insulin.

493. Analysis of plasma lipoproteins glycosylated in vitro

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Diabetic patients are particularly at risk from premature atherosclerosis which may be related to glycosylation of plasma lipoproteins. We report on the in vitro glycosylation of VLDL, LDL and the high density lipoproteins HDL_2 and HDL_3 isolated from pooled normal plasma by ultracentrifugation. Lipoproteins were incubated at 37 °C in phosphate buffer (pH 7.4) containing high concentrations of glucose or glucose-6-phosphate with sodium cyanoborohydride. Glycosylated lipoproteins showed enhanced mobility on agarose electrophoresis, LDL being the species most affected. Glucose-6-phosphate had a greater effect on electrophoretic migration than glucose. LDL fractions were subjected to amino-acid analysis and a procedure employing thiobarbituric acid to determine the extent of glycosylation. LDL incubated with glucose had reduced (−35%) levels of lysine and was more reactive on analysis with thiobarbituric acid. These data would indicate that glycosylation had proceeded via the formation of a ketoamine linkage with the lysine residues of the apoprotein. Glucose-6-phosphate-treated LDL yielded data suggesting that the binding of this moiety to the lipoprotein may involve an alternative mechanism.

494. Failure of improved diabetic control to reverse diabetic autonomic neuropathy

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The aim of this 2-year study was to measure the effects of improved control on abnormal autonomic and peripheral nerve function. Twenty insulin-treated patients, mean age 34.6 years (range 15–50 years)

were studied. Their response to intensive conventional treatment with home monitoring of blood glucose was assessed by monthly HbA_1 measurement. The initial mean ± SEM level of 13.0 ± 0.7% fell to 10.4 ± 0.4% after 3 months and to 9.3 ± 0.4% after 24 months. Eleven different neurological tests of vibration sense, pupillary and cardiovascular function were performed at 0, 3, 6, 12, 18 and 24 months yielding 1,320 test results for regression analysis. This showed that mean results remained stable in the following six tests: resting heart rate, tachycardia on passive tilting, sinus arrythmia on deep breathing, vibration sense at great toe, pupillary light reflex amplitude and dilatation to phenylephrine eyedrops. In the other five tests (resting sinus arrhythmia, Valsalva ratio, postural fall in systolic blood pressure on tilting, darkness pupil diameter and dilatation to hydroxyamphetamine eyedrops) results showed a significant deterioration. Thus, despite the achievement of a sustained improvement in diabetic control, there was no reversal of the neuropathy.

495. Glucagon-mediated C-peptide release: dose-response in Type 2 (non-insulin-dependent) diabetes

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Seven Type 2 diabetic patients, all lean, well controlled on diet alone and not receiving any medication, were given bolus injections of glucagon intravenously in the following doses: 1.0; 2.0; 5.0; 10.0 μg/kg body weight and finally 1 mg = 14.2 ± 2.2 μg/kg. Tests were performed with intervals of 3–4 days. Blood samples were obtained at 0, 2, 4, 6, 8 and 10 min for glucose and plasma C-peptide measurements (radioimmunoassay using antiserum M 1230). C-peptide peak values were seen at 4 min with the lowest doses and at 6 min with doses above 5.0 μg/kg. Area under the C-peptide response curves were (mean ± SEM): (1 μg): 1.71 ± 0.25; (2 μg): 2.87 ± 0.28; (5 μg): 3.46 ± 0.55; (10 μg): 3.45 ± 0.42 and (1 mg): 3.85 ± 0.34 nmol · l^{-1} · min^{-1}. In conclusion, a well-defined dose-response relationship between glucagon dose and C-peptide release exists in Type 2 diabetes. Maximum response was achieved with 35% of routine glucagon dose, suggesting that a reduction of dose to 1.0–1.5 μg/kg might improve the sensitivity of the glucagon-stimulation test.

496. Sorbinil reduces kidney sorbitol and fructose concentrations in experimental diabetes

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Accumulation of sorbitol and fructose have been demonstrated in the kidney and it is possible that the increased activity of the polyol pathway in kidney could play a part in renal damage in diabetes. The aim of present study was to examine the effects of an aldose reductase inhibitor (sorbinil – Pfizer UK) on the level of metabolites and on the activity of enzymes in kidney of diabetic rats. Sorbinil was administered to alloxan-diabetic rats orally as a daily dose of 10 mg/kg body weight or 40 mg/kg body weight on the day of induction of diabetes and thereafter for 6 days. After treatment with sorbinil there was a significant fall in kidney sorbitol concencentration (control 255 ± 14; diabetic 320 ± 20; diabetic + 10 mg/kg 253 ± 5; diabetic + 40 mg/kg 246 ± 10 nmol/g; mean ± SEM) and kidney fructose concentration in the same groups (174 ± 9; 257 ± 20; 213 ± 9; 187 ± 12 nmol/g). The decline in the level of glucose-6-phosphate and fructose-6-phosphate, metabolites known to be markedly increased in kidney in diabetes, was also observed after treatment with 40 mg/kg sorbinil. Reduced activity of sorbitol dehydrogenase and aldose reductase by sorbinil was also detected. It is concluded that sorbinil is an effective inhibitor of sorbitol and fructose synthesis in kidney of diabetic rats.

497. Effect of insulin antibodies on ^{123}I-insulin bioavailability in insulin dependent diabetic patients

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^{123}I-Tyr-A14-insulin (1 mCi, ^{123}I-insulin) was injected intravenously in 20 normal volunteers and in two Type 1 (insulin-dependent) diabetic patients (P1 and P2) and the biodistribution of the tracer was followed by scintillation scanning. Plasma levels of antibodies were 10 and > 20 U/l respectively in P1 and P2. P2 was insulin resistant. In normal volunteers, ^{123}I-insulin was rapidly taken up by the liver and kidneys (maximum radioactivity between 5 and 10 min). Thereafter, radioactivity rapidly decreased in both organs. In the two Type 1 diabetic patients, ^{123}I-insulin was cleared more slowly from the blood. Kidney radioactivity was much lower in P1 and undetectable in P2. In P1, maximum liver radioactivity was delayed and the slope of liver radioactivity decrease was sluggish. In P2, liver radioactivity increased until 20 min then levelled off for the following 25 min. In P1, insulin-antibody complexes were in equilibrium with free insulin, explaining decreased radioactivity uptake by the kidneys and flattened liver radioactivity profile. In P2, all injected insulin was antibody-bound and, by analogy with animal studies, was probably cleared by the Kupffer cells. Lack of free insulin explained absence of kidney radioactivity uptake. In conclusion, antibodies may have an insulin-retarding effect (P1) or a scavenging effect (P2).

498. Effect of injection site on liver uptake of ^{123}I-insulin as measured by scintillation scanning in normal rats

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Carrier-free ^{123}I-Tyr-A14-insulin (^{123}I-insulin, 50 μCi) was injected intravenously into anaesthetized rats lying on the collimator of a scintillation camera connected to a computer, to obtain kinetic data of the tracer distribution. After portal vein injection (PoVI), liver activity (LA) reached 70% of injected dose after 30 s, remained at that level for 4.5 min, then decreased with a half-life of 6 min. After peripheral vein injection (PeVI), LA reached 30% between 3 and 4 min, then decreased with the same half-life. Initial uptake of ^{123}I-insulin by the kidneys was significantly lower after PoVI (5 versus 8% of dose at 3 min) because α (hepatic extraction coefficient) insulin was retained by the liver during the first transhepatic passage and only $(1-\alpha)$ insulin reached the general circulation. Using the equation $\alpha = \frac{\text{LA after PoVI} - \text{LA after PeVI}}{1 - \text{LA after PeVI}}$ we calculated α to be 0.61. Knowing α, we also calculated LA profile after the first transhepatic passage and, using this new curve, determined the minimum (4.5 min) and average (13 min) life of insulin molecules in the liver. In conclusion, our results stress the importance of insulin injection site on hormone delivery to the liver versus peripheral tissues. This new technique allows estimation of the life expectancy of insulin molecules in the hepatocytes.

499. Continuous subcutaneous insulin infusion and exercise: the adjustment of basal and pre-meal insulin requirements

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The adjustment of basal insulin infusion rates (BR) and pre-meal insulin boluses (IB) were assessed to prevent exercise-induced hypoglycaemia. Seven male Type 1 (insulin-dependent) diabetic patients on continuous subcutaneously insulin infusion (CSII) exercised for 60 min on an ergometer at a workload of 80 W. The exercise was followed by a rest period of 6 h. Mean BR was 1.1 ± 0.2 U/h (mean ± SEM) and mean IB given at breakfast 90 min before exercise was 1.5 ± 0.1 U/12 g carbohydrate. Each patient completed five protocols (P1–P5) differing in BR and IB. In P1, 100% BR was given without exercise: normoglycaemia maintained throughout the study. In P2, 100% BR led to hypoglycaemia during and after exercise. In P3–P5, BR was discontinued during exercise; however, thereafter a BR of 100%, 50% and 75% was given in P3, P4 and P5, respectively. In P3 and P4, hypoglycaemia was observed during exercise, and mean blood glucose thereafter remained in a low range of 5 mmol/l in P3, while in P4 it increased from 4.8 ± 0.7 to 8.4 ± 1.2 mmol/l. In P5, IB was reduced by 50%: no hypoglycaemia occurred during or after exercise, and mean blood glucose increased slightly from 6.8 ± 1.4 to 7.9 ± 0.9 mmol/l. Thus, in CSII-treated diabetic patients at normoglycaemia (1) the basal rate must be discontinued during moderate exercise, and the pre-meal bolus before exercise should be reduced by 50% to prevent hypoglycaemia during exercise and, (2) late hypoglycaemia after exercise can be avoided by reducing the basal rate to 75% of its pre-exercise rate.

500. Selective insulinization of the liver by means of targeted liposomes in conscious diabetic dogs

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A variety of benefits have been ascribed to portal insulin delivery in diabetic animals. We hoped to mimic portal insulin delivery by means of encapsulating insulin in unilamellar vesicles, and targeting these vesicles (termed vesicle encapsulated insulin or VEI) to the liver by means of a digalactosyl diglyceride moiety. Conscious diabetic dogs were given peripheral infusion of insulin and VEI at a dose of 1.0 mU · kg^{-1} · min^{-1}. Plasma glucose levels were held constant by

means of an exogenous glucose infusion, and the rate of hepatic and extrahepatic glucose utilization were monitored by the arteriovenous method. The steady-state rate of extrahepatic glucose utilization was significantly less in VEI-treated animals ($n=11$) when compared with animals receiving insulin ($n=7$) 6.36 ± 1.21 versus 8.82 ± 1.61 mg·kg^{-1}·min^{-1} ($p<0.03$). No significant differences were noted in the steady-state hepatic glucose utilization rates (VEI = 3.89 ± 1.71; insulin = 1.20 ± 0.88 mg·kg^{-1}·min^{-1}). The percentage of infused glucose that was deposited in hepatic versus extrahepatic tissues was dramatically different. Insulin exhibited an 11.3% hepatic versus 88.7% extrahepatic ratio, and VEI a 35.2% hepatic versus 64.8% extrahepatic ratio ($p<0.03$). In conclusion, (1) selective insulinization of the liver can be achieved by encapsulating insulin into hepatically-targeted vesicles; (2) VEI treatment results in an increased deposition of glucose in the liver.

501. The influence of improved metabolic control on mineral metabolism and bone density.

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Type 1 (insulin-dependent) diabetes is associated with increased prevalence of osteopenia. The influence of 1 year of improved metabolic control by continuous subcutaneous insulin infusion (CSII) on bone density and mineral metabolism was studied in 10 Type 1 diabetic patients (mean age 42.5 (range 32–58) years, insulin-dependent for 24.7 (range 13–43) years). Mineral metabolism was evaluated by calcium, phosphate, alkaline phosphatase, parathyroid hormone, calcitonin, vitamin D derivatives 25-hydroxy-D_3, 1,25-dihydroxy-D_3 and 24,25-dihydroxy-D_3, and urinary calcium and phosphate excretion. Bone density was measured by computerized tomographic X-ray densitometry of L4 and expressed in Hounsfield units (HU). Improved control was confirmed by a decrease of HbA_1 (13.3 – 10.7%, $p<0.01$). After 3 months there were increases in serum calcium (from 2.36 to 2.45 mmol/l, $p<0.05$) and free 1,25-dihydroxy-D_3 (from 14 to 20 fmol/l) ($p<0.05$); and decreases in urinary calcium: creatinine ratio (from 0.55 to 0.43, $p<0.05$) and plasma alkaline phosphatase (from 82.9 to 73.0 IU/l, $p<0.01$). After 6 months parathyroid hormone had risen from 0.232 to 0.351 nmol/l, $p<0.005$). Other variables remained unchanged. Mean bone density remained stable in four and increased from mean 170 to 206 HU in two patients. Thus, the changes in mineral metabolism which occur during improved metabolic control, may be associated with a stable or increased bone density.

502. A comparative study of Visidex II and BM Glycaemie 20-800 blood glucose test strips

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The accuracy of a new blood glucose test strip – Visidex II (Ames) was compared with that of an established strip – BM 20-800 Glycaemie (Boehringer). Five medical students each tested a total of 50 blood samples with both Visidex II and BM 20-800 Glycaemie. Correlations between strip estimations and laboratory values (Yellow Springs Instruments glucose analyser) were sought using linear regression analysis. Over the blood glucose range 0– < 10 mmol/l, there was no significant difference in the performance of the two strips, and laboratory and strip values were highly correlated for both systems (n = 121; BM 20-800: y = 0.91 + 0.94, R = 0.89; Visidex II: y = 1.63 + 1.22, R = 0.80). However, at higher glucose values there was a pronounced underestimation of blood glucose with Visidex II (glucose ⩾ 10 mmol/l, $n=119$; BM 20-800: $y=0.82+1.85$, $R=0.79$; Visidex II: $y=1.05-2.96$, $R=0.72$). Furthermore, the mean deviation of strip estimations from laboratory values was significantly greater with Visidex II above 10 mmol/l (mean ± SEM difference, BM 20-800 versus Visidex II, 1.99 ± 0.17 versus 3.84 ± 0.24 mmol/l, $p<0.001$). In conclusion, Visidex II appears less accurate than BM 20-800 Glycaemie above 10 mmol/l.

503. Metoclopramide therapy in gastroparesis diabeticorum

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Gastric motor function was studied in 14 normal subjects and 12 with gastroparesis diabeticorum (GD) before and after administration of intravenous metoclopramide. Metoclopramide significantly increased the cumulative antral activity both in normal subjects and patients with GD. The number of antral contractions significantly increased after metoclopramide only in patients with GD. Fifteen patients with GD were selected for a randomized, double-blind, controlled trial of metoclopramide (40 mg daily) for 3 weeks. The mean total symptom score was significantly lower after treatment; no significant changes were obtained with placebo. It is concluded that metoclopramide is an effective agent in the treatment of the patients with GD, probably due to its action on gastric smooth muscle, thus improving gastric emptying.

504. Assessment of gestation by salivary progesterone and its relevance to the concept of early growth delay in diabetic pregnancy

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Early growth delay has been cited as a frequent abnormality in diabetic pregnancy. Confirmation or otherwise of this pattern depends upon accurate identification of the date of conception. In a group of 11 insulin-dependent diabetic women attending a pre-pregnancy clinic, daily measurements of salivary progesterone were made during the cycle in which they conceived. A clear ovulatory rise was evident in all cases. The precise date of conception was known also for one other diabetic patient who had artificial insemination by donor. Ultrasound measurement of early fetal growth in these 12 pregnancies showed no abnormality when gestational age was measured from the known date of ovulation. However, when gestational age was estimated in the conventional way from menstrual histories in these patients, an apparent 'delay' in ultrasound growth measurement was common. Even in a larger group of non-diabetics from whom patients with irregular cycles had been excluded a group showed apparent 'delay'. These findings, together with the high frequency of menstrual irregularity in diabetic subjects, suggest that apparent early growth delay in diabetic pregnancies may be attributed to an incorrect estimate of the date of conception.

505. Induction therapy of non-insulin-dependent diabetes: inducibility of glucose and drug metabolism in experimental diabetes in rats

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Therapy with medroxyprogesterone acetate (MPA) and phenobarbitone (PB) improves glycaemic control in non-insulin-dependent diabetes. Since the inducing properties of the compounds may differ, we compared the effects of MPA and PB on glucose and drug metabolism in experimental diabetes in rats. Diabetes was produced by a single injection of streptozotocin (65 mg/kg) after which MPA (100 mg/kg) or PB (75 mg/kg) was given for 7 days. Blood glucose, serum immunoreactive insulin, hepatic glucose-6-phosphatase activity and glycogen content were used as indices of glucose handling. Drug metabolism was assessed by measuring cytochrome-P450 (cyt-P450) content and NADPH cyt-P450 reductase, aryl hydrocarbon hydroxylase and 7-ethoxycoumarin O-deethylase (ECD) activities. MPA and PB increased all the measured drug metabolizing enzyme activities. Cyt-P450 content and ECD activity were more pronounced in the PB- than in MPA-treated rats. MPA and PB decreased hepatic glycogen content. Gluconeogenesis was enhanced in MPA-treated animals but remained unaltered in PB-treated animals. The compounds did not change blood glucose or serum insulin levels. The findings demonstrate that MPA and PB influence glucose and drug metabolism in diabetes. The inducing properties of the compounds are divergent, which may have significance when tailoring diabetes therapy.

506. Long-term intraperitoneal insulin administration by multiple injections

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Forty peritoneal access devices (internal vol. 3 ml) have been implanted in 23 patients with Type 1 (insulin-dependent) diabetes > 10 years duration and secondary complications. Patients injected soluble insulin (5 or 10 U) 3–4 times daily. Units of insulin entering the peritoneal cavity (Ip), units injected (Ii) and volume injected (Vi) are related by: $\frac{Ip}{Ii}=\frac{Vi/3}{Vi/3+1}$. Ip represents a 'bolus dose' and Ii-Ip is the 'basal infusion', diffusing slowly into the peritoneal cavity. Thirteen of 23 patients have been assessed for 6–25 months to a cumulative time of 17.5 patient years. Eight patients demonstrated HbA_1 values within the normal range (4·2–9·1%) and the mean HbA_1 value ($n=165$) for

all 13 patients was 8·3 ± 1%. The major complication was mesothelial over-growth obstructing the peritoneal inlet port. Improved design and materials have increased actuarial 6-month device survival from 50% to 86%. Infection was of minor consequence: four patient-days' hospitalization. Ketoacidosis did not occurre and there were 12 episodes of hypoglycaemia requiring external aid. In conclusion, this access device offers inherent control of intraperitoneal insulin delivery, glycaemic control has proved adequate and complications, initially prevalent, are declining.

507. Role of glucagon suppression in insulin treatment of the conscious diabetic dog

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In seven insulin-deficient (< 3 mU/l) depancreatized dogs, the direct and glucagon-related indirect effects of intraportal insulin infusion (350 mU · kg^{-1} · min^{-1} for 300 min; 12 ± 1 mU/l) on glucose production and gluconeogenesis were determined. Plasma glucagon was allowed to fall (from 314 ± 94 to 180 ± 63 pg/ml) in response to insulin for 150 min and was then replaced intraportally (2.6 ng · kg^{-1} · min^{-1}; 310 ± 51 pg/ml) for 150 min. Glucose production and gluconeogenesis were determined using the arterio-venous difference technique and ^{14}C-alanine infusion (for gluconeogenesis). In the control period and after 150 and 300 min respectively, plasma glucose was 24.7 ± 2.3, 14.1 ± 1.2, and 16.2 ± 1.0 mmol/l, net hepatic glucose output was 30.2 ± 2.9, 0.0 ± 2.5, and 21.0 ± 5.3 mmol · kg^{-1} · min^{-1}, plasma alanine was 442 ± 89, 665 ± 101, and 331 ± 42 μmol/l, hepatic fractional extraction of alanine (portion of delivered load extracted) was 0.41 ± 0.10, 0.24 ± 0.06, and 0.45 ± 0.04, blood lactate was 801 ± 145, 1089 ± 183, and 680 ± 121 μmol/l, and lactate hepatic fractional extraction was 0.32 ± 0.09, 0.04 ± 0.03, and 0.33 ± 0.13. Alanine conversion to glucose had fallen by 83 ± 14% at 150 min, but returned to baseline by 300 min (99 ± 23% of control). In conclusion, 70% and 100% of the fall in hepatic glucose production and gluconeogenesis respectively caused by insulin is mediated through a fall in glucagon.

508. Human proinsulin: acute hypoglycaemic effects and response to a subsequent meal

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The availability of biosynthetic human proinsulin has enabled us to study its acute hypoglycaemic action, to assess its effect on the carbohydrate intolerance which follows acute hypoglycaemia and to compare its activity with that of insulin. Human proinsulin (Eli Lilly), insulin (Humulin S) or saline as control were administered intravenously to eight healthy non-obese subjects. The dose of insulin was 0.15 U/kg; proinsulin was given on separate occasions in either estimated equi-hypoglycaemic (0.96 mg/kg) or equimolar doses (0.0375 mg/kg). There was no significant difference between insulin and equi-hypoglycaemic proinsulin in the clinical response or the degree or duration of hypoglycaemia (mean ± SEM plasma glucose nadir 1.3 ± 0.13 mmol/l for insulin and 1.8 ± 0.18 mmol/l for proinsulin). Equimolar proinsulin produced only a slight fall in plasma glucose (3.1 ± 0.2 mmol/l; control 4.2 ± 0.2 mmol/l). Following a standard lunch given 180 min after insulin/proinsulin, all subjects demonstrated hyperglycaemia (peak plasma glucose 9.0 ± 0.47 mmol/l for insulin, 8.0 ± 0.65 mmol/l for equi-hypoglycaemic proinsulin and 7.1 ± 0.2 mmol/l for control). The doses of proinsulin and insulin used in this study result in similar degrees of hypoglycaemia and posthypoglycaemic carbohydrate intolerance.

509. Effect of an aldose reductase inhibitor, ICI 128,436, on abnormalities in sensory function in the diabetic rat

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Nerve sorbitol accumulation has been implicated in the development of diabetic neuropathy. The effects of an aldose reductase inhibitor, ICI 128,436 (25 mg · kg^{-1} · day^{-1} in the diet), upon the response to paw pressure in the diabetic rat has been investigated during the first 30 days after streptozotocin injection (60 mg/kg intravenously). At 9 days, the weight applied to the paw necessary to induce foot withdrawal in diabetic control rats (429 ± 37 g) was greater than that in non-diabetic age-matched control rats (301 ± 19 g, $p < 0.01$). Treatment with ICI 128,436 ameliorated this defect (diabetic rats + ICI 128,436; 358 ± 41 g, non-diabetic rats + ICI 128,436; 340 ± 38 g). This sensory abnormality and its improvement by ICI 128,436 treatment persisted for up to 21 days after streptozotocin injection. After 21 days, diabetic rats became more responsive to paw pressure than non-diabetic controls (27 days: diabetic controls: 245 ± 22 g, non-diabetic controls: 366 ± 25 g, $p < 0.01$). The effect of ICI 128,436 upon this sensory defect was more variable. Thus streptozotocin-diabetic rats develop abnormalities in sensory function, the nature of which depends upon the duration of diabetes. Treatment with ICI 128,436 significantly reduces the severity of the early sensory defect but has a less consistent effect upon the later changes.

510. Changes in autonomic nerve function and glomerular filtration rate in Type 1 (insulin-dependent) diabetes

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Twenty-four patients with short (group A) and 19 with a long duration of diabetes (group B) were followed for 5–7 years, and autonomic nerve function was evaluated by the heart rate reaction to deep breathing (E/I ratio) and to tilting (acceleration and brake indices) and glomerular filtration rate (GFR) by the ^{51}Cr-EDTA clearance method. At follow up, 10 patients in each group showed a decrease in GFR. In the primary study, group A patients with decreases in GFR showed a lower mean brake index (7.0 ± 1.7) than patients without (14.1 ± 2.3; $p < 0.05$) and control subjects (18.7 ± 2.5; $p < 0.01$). The mean brake index was also lower in group B patients with decreases in GFR (4.9 ± 1.4) than in those without (14.5 ± 3.1; $p < 0.05$) and control subjects (14.2 ± 1.8; $p < 0.01$). However, those patients also showed a lower mean E/I ratio (1.09 ± 0.02) and acceleration index (10.4 ± 1.8) than those without (1.20 ± 0.04 and 17.3 ± 2.6; $p < 0.05$) and control subject (1.23 ± 0.02; $p < 0.001$ and 17.0 ± 1.5; $p < 0.05$). Nevertheless, in both groups, a low brake index pointed to deterioration in GFR. Furthermore, a high brake index (≥ 15) only occurred in patients without a decrease in GFR and not in those with (11 out of 23 versus 0 out of 20; $p < 0.001$).

511. Regulation of insulin release and ^{45}Ca uptake by rat insulinoma cells maintained in tissue culture

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Acute effects of nutrients, hormones and drugs on transplantable rat insulinoma cells were examined after 2–3 days culture in RPMI-1640 (11.1 mmol/l glucose) to eliminate necrotic cells and counter prior hypoglycaemia. At Ca^{2+} (2.56 mmol/l), insulinoma cells (> 95% viability; ~156 μg insulin/10^6 cells) released 49–139 ng insulin/10^6 cells during 60 min incubations with uptake of 1.0–2.4 nmol ^{45}Ca/10^6 cells. Glucose (16.7 mmol/l) alone or in combination with theophylline (5 mmol/l) failed to modify insulin release or ^{45}Ca uptake. Insulinoma cells were similarly unresponsive (at 11.1 mmol/l glucose) to arginine (10 mmol/l), leucine (20 mmol/l), 2-ketoisocaproate (10 mmol/l), glucagon (1 μmol/l), somatostatin (1 μmol/l), adrenaline (10 μmol/l), noradrenaline (10 μmol/l), diazoxide (0.44 mmol/l) and cyproheptadine (10 μmol/l). Responsiveness to 10 μmol/l acetylcholine (64% and 15% stimulation of uptake and release) and 1 μmol/l GIP (22% stimulation of release) was retained. Cytochalasin B (10 μmol/l), colchicine (50 μmol/l) and vinblastine (50 μmol/l) increased insulin release by 11–15%, with the latter agents evoking 27–44% elevations of ^{45}Ca uptake. However, K^+ (25 mmol/l) and drugs (20–50 μmol/l verapamil and D-600) with established effects on transmembrane Ca^{2+} fluxes failed to affert insulin release or ^{45}Ca uptake. Furthermore, insulin release was independent of extracellular Ca^{2+} over the range 0–20.4 mmol/l. The results indicate that rat insulinoma cells exhibit a generalized defect in the regulation of insulin release. The underlying lesion is proximal to the microtubule-microfilament system and appears to concern enhanced sensitivity to and disturbances in the regulation of cytoplasmic Ca^{2+}.

512. Glucose stimulation of islet cell replication is a late event in fetal development of the rat

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Since islet cell hyperplasia of diabetic fetopathy has been difficult to reproduce in the rat, glucose regulation of islet cell replication has been studied in rat fetuses of different gestational ages. Islets were isolated from rat fetuses at days 18, 20 and 22 of gestation and tissue cultured at low (2.7 mmol/l) and high (16.7 mmol/l) glucose concentrations. Some cultures were supplemented with essential amino-acids, growth hormone or triiodothyronine. Islet cell replication was measured autoradiographically after incubation with tritiated thymidine

and insulin release by estimations of insulin accumulated in the culture medium. Islets from 22-day-fetuses were stimulated to replicate by either glucose or amino-acids. Islets from younger fetuses responded only to amino-acids. At all ages, both glucose and amino-acids stimulated insulin release. Growth hormone, but not triiodothyronine, stimulated replication and insulin release at all ages. The results indicate that glucose-stimulated islet cell replication develops at the end of pregnancy and that other factors may regulate islet growth prior to this period. The late occurrence of glucose-stimulated islet growth may explain the difficulties in producing islet cell hyperplasia in the offspring of diabetic rats.

513. Short-term effects of two strains of EMC virus on the biochemical functions of mouse islets

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Encephalomyocarditis (EMC) virus is pathogenic in mice, but whereas the M strain produces diabetes in susceptible mice, the E strain does not. In this study, the short-term effects of the E and M strains of virus on the biochemical functions of mouse islets in vitro were compared. Collagenase-isolated islets from adult, male DBA/2 mice were infected with TCD_{50} doses of EMC-M and EMC-E. After 24 h in culture the responses of the islets to glucose (2 and 20 mmol/l) were measured. At glucose (2 mmol/l) there was an increase in insulin release from islets infected with EMC-M when compared with the response in control islets or EMC-E infected islets ($p < 0.05$). By contrast, secretion was greatly inhibited in the MC-M infected islets at glucose (20 mmol/l) ($p < 0.05$). Proinsulin biosynthesis was diminished in islets infected with either strain of virus at glucose (20 mmol/l) ($p < 0.01$), and in addition in islets infected with EMC-M there was a reduction in total protein synthesis ($p < 0.01$). A secretory deficit appears therefore in islets infected with a diabetogenic strain of EMC virus, and it is probably accompanied by leakage of insulin at low glucose concentrations.

514. Neonatal and maternal complications of pregnancy in women with a normal oral glucose tolerance test: correlation with third trimester glucose tolerance

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The level of glucose intolerance associated with increased materno-fetal complications of pregnancy is debated. We tested 249 women in the third trimester of pregnancy with a standard oral glucose tolerance test (GTT) (100 g). In all women without previous evidence of diabetes, the oral GTT was normal (O'Sullivan criteria). We divided them in to three groups on the basis of the 120 min glucose level): group A (< 5.56 mmol/l), group B (5.56–6.61 mmol/l), group C (6.67–8.89 mmol/l). The incidence of macrosomia, perinatal mortality, prematurity, congenital malformations, toxaemia and Caesarean section were also evaluated. We found a highly significant correlation between macrosomia and the 120 min oral GTT level (10%, 15%, 27% in groups A–C, respectively). Moreover, we found a significant correlation between fetal malformations and the 120 min oral GTT level (0.6%, 3.4%, 5% in groups A–C, respectively). An highly significant correlation was found between the incidence of toxaemia and Caesarean section considered together and the 120 min oral GTT level (20%, 26%, 40% in groups A–C, respectively). In considering group A versus groups B + C, we found a significant increase of incidence of macrosomia (9 versus 20%), malformations (0.6% versus 4%), toxaemia and Caesarean section (19% versus 32%). These results suggest that greater attention should be paid to pregnant women whose 120 min oral GTT levels are between 5.56 and 8.89 mmol/l, stressing the opportunity of defining a class of impaired gestational glucose tolerance.

515. Fitting the results of the oral glucose tolerance tests to an empirical equation

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The recommendation of a 75 g glucose-load for an oral glucose tolerance test, evaluated by the two criteria published recently, has stimulated us to develop a method for comparing the results obtained with different glucose loads (50, 75 and 100 g). The plasma concentrations of glucose (G; o-toluidine method) and insulin (radioimmunoassay, IRI) were measured at several times. Ours, as well as other published results, fit well to the equation:

$$Y = C_1 \exp(-K_1 t) + C_2 t \exp(-K_1 t) - C_1 \exp(K_2 t) \quad (1).$$

Y represents the increments of G or IRI with respect to basal values and the equation satisfies the limiting conditions ($t = 0$ and ∞). The exponents (K_1 and K_2) vary within a narrow range and the coefficients (C_i) show ample variation. The equation allowed the calculation of the initial increasing rates of G and IRI which did not show any significant difference for different loads (3.65 mg/min, G; 3.59 μU/min, IRI). On the contrary, the areas delimited by G and IRI increased significantly according to the glucose load. The differences were more marked when 50 and 100 g loads were compared. The differences between areas calculated for 75 and 100 g loads were not significant. From the known values of K_1 and K_2, initial increasing rates of G and IRI, glucose and insulin areas, the C_i coefficients may be calculated and used to obtain a corresponding theoretical model of each oral glucose tolerance test.

516. Evidence of a dual effect of glucose on $^{86}Rb^+$ fluxes in isolated rat islets

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We have investigated the effect of glucose concentration on islet uptake of $^{86}Rb^+$ in excess of (^{3}H)-sucrose space. Collagenase isolated islets were pre-incubated (37 °C) for 45 min in Krebs-Ringer bicarbonate (6 mmol/l K^+), buffered with 10 mmol/l Hepes (pH 7.4), containing 2 μmol/l (^{3}H)-sucrose and varying glucose concentrations. They were then incubated for various lengths of time, at the same glucose conentrations, with 20 μmol/l ^{86}RbCl. Radioactivity was measured in islets after centrifugation through an oil-layer. – $^{86}Rb^+$-uptake at 5 mmol/l glucose was linear for 10 min and reached isotopic equilibrium after 120 min. Glucose (20 mmol/l) induced a decrease of the initial uptake and turnover rate of islet $^{86}Rb^+$ pool, whose size increased by almost 100%. Comparison of different glucose concentration (0, 3, 5 and 20 mmol/l) revealed that both initial (5 min) and near-equilibrium (120 min) uptake of $^{86}Rb^+$ were significantly decreased and increased, respectively, by 5 and 20 mmol/l glucose. Ouabain decreased $^{86}Rb^+$ uptake at either 3 or 20 mmol/l glucose in a dose (0.1 or 1 mmol/l) dependent manner. At 1 mmol/l it reduced $^{86}Rb^+$-uptake (120 min) to very low values, abolishing the difference between 3 and 20 mmol/l glucose. The results suggest that glucose induces an inhibition of both influx (electrogenic Na^+-pump?) and efflux rate of K^+. Both glucose effects may contribute to membrane depolarization of β cells.

517. The relationship between somatostatin and diazoxide induced-insulin suppression and ultrastructural findings in functioning insulinomas

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Since both somatostatin and diazoxide have been reported to reduce to a different extent the insulin release from functioning islet β-cell tumours, the relationships between insulin suppression and ultrastructural findings of the neoplasia have been investigated in four patients suffering from single insulinoma. Blood glucose levels were kept constant at 4.4 mmol/l using a closed-loop insulin-infusion device (Biostator, Miles). Somatostatin (250 μg) was injected intravenously as a bolus followed by a constant infusion of 250 μg/h for 2 h. During the second hour, 600 mg of diazoxide were added to the infusion. Basal plasma insulin levels ranged from 30 to 100 mU/l, while glucose requirement was 170 ± 16.8 mg/min at the steady state and decreased slightly during drug administration (163 ± 39 mg/min). In the two patients with tumours containing cells with typical secretory granules, plasma insulin levels were suppressed by at least 80% during somatostatin infusion, whereas a maximum suppression by 30% was observed in the patients whose tumours were composed of cells with atypical granules. No difference was noted when diazoxide was added to the infusion. Our data demonstrate that glucose requirements and basal insulin levels are similar in the different types of insulinoma, while somatostatin induced-insulin suppression may be related to the ultrastructural findings.

518. Dissociation of very low density lipoprotein triglyceride and apolipoprotein B metabolism in Type 2 (non-insulin-dependent) diabetes: relation to glycaemia

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To assess the mechanism of diabetes-induced changes in plasma lipoproteins, lipoprotein composition and VLDL metabolism were studied in six obese (177 ± 20% ideal weight) Type 2 diabetic Pima Indians

(three women, three men, mean age 32 ± 5 years) before and after tolazamide therapy for one month. VLDL-triglyceride (TG) and VLDL-apolipoprotein B (apoB) kinetics were investigated by injection of ^{3}H-glycerol and autologous ^{125}I-VLDL, and the data were analyzed using a multicompartmental model. Fasting glucose and glycosylated haemoglobin were 13.2 ± 1.2 versus 7.7 ± 0.7 mmol/l and 12.7 ± 0.8 versus 8.8 ± 0.5% before and after treatment, respectively. Improvement of glycaemic control was followed by a significant fall of VLDL-TG (1.6 ± 0.3 versus 1.2 ± 0.3 mmol/l, $p < 0.01$), VLDL-apoB (96 ± 16 versus 72 ± 11 mg/dl, $p < 0.05$) and LDL-cholesterol (3.4 ± 0.4 versus 2.8 ± 0.3 mmol/l, $p < 0.05$), and a decrease in the ratio of VLDL-TG/VLDL-apoB. HDL cholesterol and subfractions did not change significantly after treatment. VLDL-TG transport (synthesis) decreased markedly after treatment (606 ± 68 versus 408 ± 76 mmol/day, $p < 0.01$), whereas fractional catabolic rate of VLDL-TG did not change significantly. In contrast, VLDL-apoB transport was not changed significantly after therapy (1561 ± 202 versus 1474 ± 360 mg/day), but fractional catabolic rate of VLDL-apoB increased (5.5 ± 0.8 versus 6.4 ± 0.7 per day, $p < 0.01$). It is concluded that treatment of glycaemia in Type 2 diabetes has independent effects on the metabolism of VLDL-triglyceride and VLDL-apoB.

519. An examination of the physiological role of dimerization and negative co-operativity, using the biological properties in vivo of despentapeptide and deshexapeptide insulins

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The C-terminal region of the B chain is essential for dimerization and expression of negative co-operativity. Despentapeptide insulin (DPI; B26–30 deleted) is reported to have ≃20% potency in vitro and ≃ 15% retention of negative co-operativity. Deshexapeptide insulin (DHI; B25–30 deleted) has < 10% retention of negative co-operativity. We have examined kinetics and potency of these analogues in vivo in greyhounds using stepped-infusion technique, together with in vitro lipogenesis and binding potencies in rat adipocytes. Both analogues exerted a hypoglycaemic effect indistinguishable from that of insulin, but metabolic clearance rates were reduced (insulin = 19.1 ± 0.9; DPI = 9.7 ± 0.8; DHI = 6.4 ± 0.6 ml · min^{-1} · kg^{-1} at plasma concentration 0.5 nmol/l). Hypoglycaemic potencies relative to insulin calculated using plasma concentration rather than dose were: DPI – 40%, DHI – 31.3%. $T_{½}$ was prolonged for both analogues (DPI = 7.3 ± 0.5, DHI = 9.1 ± 0.2 min). Urinary clearance rates were similar to that of insulin. In vitro potencies were lipogenesis: DPI = 19.9 ± 0.3%, DHI = 19.9 ± 1.5%; binding: DPI = 22.6 ± 7.8%, DHI = 16.5%. Despite impaired negative co-operativity and inability to dimerise, these analogues on a dose-related basis are fully potent in vivo. Metabolism and in vitro potencies are reduced. In this respect, they resemble other analogues with modifications which reduce receptor affinity without impairing dimerization or negative co-operativity. These results do not suggest a physiological role for insulin dimerization or negative co-operativity.

520. Biological activity of despentapeptide insulin in human and rat adipocytes and rat hepatocytes

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Highly purified insulins tend to precipitate in portable infusion systems. The biological behaviour of insulin analogues which do not aggregate is therefore of interest. Despentapeptide insulin (DPI), an analogue of known crystalline structure, remains strictly monomeric even at high concentration. We have compared the insulin binding and action of DPI and unmodified insulin in human and rat adipocytes and insulin binding to rat hepatocytes. DPI had a lower affinity of binding to human adipocytes (half-maximum displacement at 89 ± 4 versus 20 ± 2 × 10^{-10} mol/l; $p < 0.001$), to rat adipocytes (72 ± 11 versus 11 ± 2 × 10^{-10} mol/l; $p < 0.05$) and to rat hepatocytes (38 ± 9 versus 17 ± 3 × 10^{-10} mol/l; $p < 0.05$). Scatchard analysis suggested that binding to high affinity receptors was predominantly affected. DPI was less potent than unmodified insulin in stimulating glucose transport in rat adipocytes (half-maximal stimulation at 200 ± 61 vs 47 ± 18 × 10^{-11} mol/l; $p < 0.05$) whereas in human adipocytes no significant difference could be demonstrated (half-maximal stimulation at 4.48 ± 0.29 versus 2.72 ± 0.51 × 10^{-11} mol/l; $p < 0.1$). Thus absence of the five C-terminal amino-acids from the insulin β chain did not affect the sensitivity of human adipocytes to DPI stimulation. The reduced receptor affinity of DPI was reflected in reduced potency in rat adipocytes but not in human adipocytes, suggesting differences in the binding-action linkage in adipocytes of the two species.

521. Dose-response effects in vivo of insulin on the metabolism and production of glucose in lean and genetically obese *(fa/fa)* rats

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The action of insulin on glucose metabolism and hepatic glucose production was studied in vivo over a wide range of insulin concentrations in lean and obese rats, using the euglycaemic clamp technique. Glucose metabolism (GM): the insulin concentration eliciting half-maximal stimulation of GM was 15 ng/ml in lean and 140 ng/ml in obese animals. Maximal GM was 6.8 mg/min in lean, markedly decreased in obese (3.6 mg/min) and obtained at insulin concentrations of 90 and 215 ng/ml for lean and obese rats, respectively. Hepatic glucose production (HGP): HGP was totally suppressed at insulin concentration of 15 ng/ml (lean) and 210 ng/ml (obese). The hormone concentration needed to obtain half-inhibition of HGP was 9 ng/ml for the lean and 140 ng/ml for the obese rats. These results show a marked in vivo insulin resistance in obese rats: in peripheral tissues, a decreased sensitivity and decreased responsiveness of insulin action was observed. In the liver the sensitivity, not the responsiveness, of the hormone was altered. However, although insulin could suppress hepatic glucose production of obese rats, such an effect was obtained only at extremely high values, far beyond a physiological range.

522. Insulin resistance in Type 1 (insulin-dependent) diabetes: evidence for defective suppression of endogenous proteolysis during multiple euglycaemic insulin-clamp studies

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Insulin-mediated glucose disposal is impaired in Type 1 diabetes. To determine whether an impairment also exists for amino-acid metabolism, five diabetic and five normal subjects were infused with 4,5-(^{3}H)-L-leucine and 1-(^{14}C)-αketoisocaproate (KIC). Leucine + KIC) flux, interconversions, leucine-oxidation and incorporation into protein(s) (PS) were estimated at steady-state using a non-compartimental model. Normal and diabetic subjects were studied in euglycaemia at basal and various insulin concentrations (30, 100 and 1500 mU/l). Basal leucine (132 ± 5 versus 119 ± 11 μmol/l), KIC (39 ± 2 versus 34 ± 2 μmol/l), leucine + KIC flux (1.76 ± 0.15 versus 2.10 ± 0.15 μmol · kg^{-1} · min^{-1}), leucine-oxidation (0.46 ± 0.14 versus 0.51 ± 0.08), PS (1.30 ± 0.14 versus 1.59 ± 0.12) and interconversions were similar in normal and diabetic subjects, respectively. At maximal insulin levels, leucine + KIC flux (1.33 ± 0.08 versus 1.74 ± 0.11, $p < 0.02$), PS (1.13 ± 0.07 versus 1.43 ± 0.09, $p < 0.05$) and interconversions ($p < 0.05$) were lower in normal than in diabetic subjects, while leucine (50 ± 4 versus 64 ± 7), KIC (16 ± 2 versus 19 ± 1) and leucine-oxidation (0.20 ± 0.03 versus 0.30 ± 0.07) were not different. Insulin increased leucine clearance similarly in both groups. In summary, diabetic patients exhibited a reduced responsiveness to insulin as regards leucine + KIC flux, PS and leucine/KIC reversible transamination. In conclusion, assuming that leucine represents an index of endogenous protein metabolism, a resistance to insulin-mediated suppression of endogenous proteolysis, PS and leucine/KIC transamination is suggested in Type 1 diabetes.

523. Predictors of retinopathy and its progression during intensive glycaemic control: a multivariate statistical analysis

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We studied 70 Type I (insulin-dependent) diabetic patients participating in a multi-centre trial of control and complications to analyze associations of predictor variables with baseline levels and 8–10 month changes in retinopathy. Patients with non-proliferative retinopathy were randomly assigned to insulin pump ($n = 35$) and conventional therapy ($n = 35$). Predictor variables (sex, age, duration of disease, blood pressure, plasma glucose, HbA_1, M-values, cholesterol, triglycerides, creatinine clearance and retinopathy (fundus photography and fluorescein angiography) were measured periodically. For the en-

tire group, baseline retinopathy was positively correlated ($p<0.05$) with baseline blood pressure, glucose, HbA_1, cholesterol, and duration of disease and negatively correlated with creatinine clearance. Conversely, during treatment, progression of retinopathy was negatively correlated ($p<0.05$) with absolute treatment levels of glucose, HbA_1, M-values, cholesterol, and with changes during treatment in glucose and triglycerides. Significant dependencies ($p<0.05$) also existed among predictor variables. Two-group multivariate classification analysis of retinopathy (improved or unchanged versus progression) indicated lower glucose as the best predictor of worsening of retinopathy ($p=0.047$), correctly classifying 71% of patients with positive progression. In conclusion, associations among predictors and retinopathy during improved glycaemic control strikingly differ from those at baseline suggesting that the relationship between glycaemia and its control and retinopathy is not a simple one.

524. Neuropsychological performance at different blood glucose concentrations in Type 1 (insulin-dependent) diabetic patients

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Neuropsychological skills were assessed at normal, subnormal, and low blood glucose concentrations in Type 1 diabetic patients. We studied 16 fasting, right-handed male patients, mean age 28 years (20–46 years) and duration of diabetes 12 years (4–28 years), insulin dose 0.68 IU/kg body weight (0.31–1.27). The neuropsychological testing (NPT) was carried out at four blood glucose levels: (A) 6.3 ± 0.1 mmol/l (mean ± SEM), (B) 2.9 ± 0.1, (C) 1.8 ± 0.03 by means of an intravenous insulin infusion, and (D) 6.1 ± 0.1 after intravenous glucose. The total score in NPT deteriorated from A to B, A to C, and B to C, whereas improvement was seen from C to D (all $p<0.01$). The results of NPT at A and D were not significantly different ($p>0.05$). The results were not due to single-subject changes, but consistent for the whole group of patients. The patients' time experience changed during hypoglycaemia so that time elapsed at low blood glucose concentration (C) was underestimated by the patients compared with B and D (both $p<0.05$). None of the patients expressed symptoms of hypoglycaemia at A or B, 12 out of 16 expressed symptoms at C, and one at D. The results demonstrate deterioration of neuropsychological skills in Type 1 diabetic patients already at a subnormal blood glucose concentration which none of the patients recognized as being hypoglycaemic.

525. Diabetes and its complications in Cyprus

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Complications, family history, duration, treatment and control were examined in 220 Cypriot Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetic patients. Positive family history was found in 48% of the males and 41% of the females. Complications were much higher in all diabetic patients compared with the World Health Organisation published figures. Cardiovascular involvement was seen in 50% of Type 1 and up to 65% in Type 2 diabetic patients. Retinopathy was present in 25% of Type 1 and in 30% of Type 2 diabetic patients. Renal involvement was recorded in 25% of Type 1 and 10% of Type 2 diabetic patients and up to 35% of patients in both groups had some degree of neuropathy. Mortality was excessive compared with published figures. A difference in complications in those with family history was seen and Type 2 diabetic patients were much more affected by complications than one would expect for this group. Only 55% of those who should be on insulin were actually receiving it. Control was poor in over 60% of the cases and fair in the remainder. It is postulated that partly poor control and partly genetics are responsible for such high rates of involvement in diabetic patients in Cyprus.

526. Frequency of biochemical hypoglycaemia at different blood glucose levels in conventionally and pump-treated Type 1 (insulin-dependent) diabetic patients

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The frequency of day-time biochemical hypoglycaemia (blood glucose < 3 mmol/l) was assessed in Type 1 diabetic patients on conventional treatment (CT; $n=79$) and on continuous subcutaneous insulin infusion (CSII; $n=21$). Patients collected and mailed to the laboratory pre- and post-prandial as well as bed-time capillary blood in Capileths from 3 to 20 days at random. The profiles were divided into classes of 1 mmol/l width according to the median blood glucose concentration (MBG) as the blood glucose (BG) did not show a normal distribution. The frequency of hypoglycaemia did not differ between CT and CSII in any class of MBG from 2 to 10.9 mmol/l. MBG showed a highly significant curvilinear relation with the relative frequency of hypoglycaemia: percentage hypoglycaemia $=365\times MBG^{-2.2}$ ($r=-0.97$, $p<0.0001$), i.e. the risk of BG < 3 mmol/l is 33% when MBG = 3 mmol/l and 10% and 2.5% when MBG is 5 and 10 mmol/l, respectively. Pre-prandial hypoglycaemia was more frequent than post-prandial ($p<0.01$) and evenly distributed during the day on CSII, whereas in patients on CT pre-lunch hypoglycaemia was four times more frequently than pre-breakfast and -dinner ($p<0.0001$). It is concluded that the frequency of biochemical hypoglycaemia is related to the median blood glucose concentration and not to whether insulin is given conventionally or by CSII.

527. Reduced cardiac output following improved metabolic control in Type 1 (insulin-dependent) diabetic patients

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Cardiac performance was assessed by echocardiography before and after improved metabolic control in eight patients with Type 1 diabetes (one female and seven males, age 21–40 years). Six patients had newly diagnosed diabetes. Two patients with duration of diabetes for 7 and 11 years were admitted because of malregulation. At the first echocardiographic examination all patients had ketonuria and hyperglycaemia (17.7 ± 2.9 mmol/l, mean ± SD). Metabolic control was improved during 5–7 days by multiple insulin injections (blood glucose 8.4 ± 2.3 mmol/l) and the echocardiographic examination was repeated. Following improved metabolic control, systolic blood pressure and heart rate decreased from 126 ± 18 to 113 ± 8 mmHg ($p<0.05$) and 73 ± 10 to 60 ± 7 beats/min ($p<0.01$), respectively. From systolic diameter, diastolic diameter and ejection time the following parameters and changes were calculated. Fractional shortening decreased by $14.4\pm11.6\%$ ($p<0.05$), mean rate of circumferential fibre shortening by $24.3\pm12.5\%$ ($p<0.05$) and cardiac output by $26.6\pm12.1\%$ ($p<0.05$). Our findings suggest that poorly controlled diabetes is associated with a cardiac hyperkinetic state with increased cardiac output, which can be rendered normal by improved metabolic control.

528. Modulation of insulin binding characteristics of isolated human blood cells after storage at 0 °C

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The use of isolated human blood cells for insulin binding studies in metabolic diseases is a widespread and accepted method. To evaluate rapidly occurring changes at the receptor level after specific treatment or conditions, such binding studies need to be carried out more than once in a day. To eliminate interassay variation we studied the influence of storage of isolated blood cells at 0 °C. Isolation and binding procedures were performed using standard methods (Bøyum, DeMeyts, Gambhir). Isolated erythrocytes (ERY) and mononuclear cells (MNC) were maintained for 24 h in assay buffer at 0 °C. Insulin binding studies were done at 0, 4 and 24 h after isolation. Isolated ERY showed no change in their insulin binding characteristics (maximal specific binding, receptor number, half-maximal inhibition dose). On the other hand, MNC revealed remarkable changes: the maximal specific binding increased by 56% ($p<0.05$), the half-maximal inhibition dose also increased (135%, $p<0.01$), while at the same time the number of receptors per cell rose by 500% ($p<0.01$). In conclusion, this shows that human ERY do not change insulin binding characteristics after 24 h preservation, while binding to MNC results in remarkable changes. Therefore, insulin binding studies on MNC must be performed immediately after isolation.

529. Follow-up of oral glucose tolerance and fasting blood glucose after 5, 10 and 15 years

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The prognostic value with respect to later development of diabetes of the 2 h-value during an oral GTT (risk class 6.67–9.94 mmol/l = IGT) was compared with the prognostic value of the fasting blood glucose (FBG) (risk class 5.56–6.61 mmol/l). Of 686 first-degree relatives of Type 2 (non-insulin-dependent) diabetic patients having received a 75 g-oral GTT in 1967, 445 were retested in 1972, 350 in 1977 and 252 in 1982. By WHO criteria 427 of the initially tested probands were normal, 170 had IGT and 89 were diabetic. In 1982 information regarding glucose metabolism was obtained in 575 probands. Test pa-

rameters for the 2-h value and for FBG (numbers in parentheses) after 5, 10 and 15 years were as follows: prevalence of diabetes 8.2%, 14.4%, 22.7%; positive predictive value 20.1% (14.9%), 28.7% (21.2%), 39% (33.5%); 100% – negative predictive value 3.8% (5.2%), 8.8% (11.3%), 16.2% (17.8%); sensitivity 65.6% (56.1%), 55.7% (45.7%), 49.1% (46.5%); specificity 76.6% (71.4%), 76.7% (71.5%), 77.4% (73%). Conclusions: the 2-h value is a slightly better predictor of later diabetes than FBG. For both parameters the prognostic value decreases with increasing time of observation, but even after 5 years is is only low. A normal glucose tolerance cannot be regarded as a good criterion to exclude later diabetes.

530. Small vessel disease in progressive diabetic neuropathy with good metabolic control

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Sural nerve biopsy was performed in 11 diabetic patients with severe progressive motor neuropathy, and good blood glucose control; one patient with painful neuropathy developed a third nerve cranial palsy. Electron microscopy showed endothelial cell hyperplasia in small vessels in all cases, and seven showed plugging of the vascular lumen by degenerate cellular material. It is postulated that endothelial cells desquamate and occlude more peripheral vessels at a point of narrowing. In one case the lumen of a vessel was occluded by thrombus and electron-microscopical examination showed a vessel occluded by degranulated platelets. In teased nerve fibre preparations of the patient with painful neuropathy and a third cranial nerve palsy, multiple small foci of demyelination and axonal degeneration were seen within otherwise normally myelinated nerve fibres. This pattern of nerve fibre damage strongly suggests a vascular aetiology. Electrophysiological studies showed a pattern of denervation which was not symmetrical and distally predominant in some patients, suggesting that the neuropathy at least in part relates to multiple small infarcts.

531. Ultrastructural modifications in the pineal gland of diabetic and glucagon-treated rats

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To determine the role of glucagon on pineal gland activity, groups of male Sprague-Dawley rats were treated either with glucagon (GR; 0.5 mg/kg by subcutaneous injection for 45 days) or with streptozotocin (DR; 50 mg/kg intravenously) and sacrificed 30 days after drug administration. Rats were subjected to a daily light-darkness cycle. In GR and DR, dramatic increases in both dense lisosome and serotonin bodies in the cytoplasm of pinealocytes were observed. We also observed the presence of 'mitochondria-lipoid-dense bodies', fusion of the serotonin bodies to lipid droplets and the exocytosis of these into the pericapillary space. The serotonin granules were identified by cytochemical techniques (chromaffin reaction and ZIO) and immunocytochemical techniques at electron microscope level, using anti-serotonin serum and gold particles with 40 nm diameter as markers. In addition HIOMT activity in the pineal glands of control and glucagon perfused rats (0.5 mg/kg for 30 min) was determined. The enzyme activity of glucagon-treated rats (30.3 ± 10.7 pmol/mg) showed a significant increase ($p < 0.05$) with respect to the control group (20.5 ± 12.2 pmol/mg). Similar results were obtained when pineal glands were cultured with different glucagon concentrations. These results suggest that exogenous and/or endogenous glucagon directly modifies the synthesis of monoamines by the pineal gland.

532. Simultaneous pancreas plus kidney transplantation in uraemic Type 1 (insulin-dependent) diabetic patients: 6 years experience

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Between September 1978 and March 1984 36 Type 1 diabetic patients, affected by end-stage renal disease due to diabetic nephropathy, received primary double simultaneous pancreas plus kidney transplants from cadaveric donors. On 31 March 1984, 20 patients (55%) were alive; 14 (39%) with both renal and pancreatic grafts functioning, four (11%) with only the renal graft functioning and one (3%) with only the pancreatic graft functioning. The cumulative patient survival was 77, 67, 66 and 54% at 3, 6, 12 and 24 months respectively; renal graft survival was 77, 66, 50 and 37% and pancreatic graft survival was 65, 54, 40 and 29%. Altogether 21 pancreatic failures occurred: 11 were due to the death of the patient, five to graft vascular thrombosis, four to rejection. When successful pancreatic transplantation led to insulin independence and correction of diabetic metabolic abnormalities, HbA_1 decreased from pre-transplant values of 10.6% ($n = 15$) to 6.7% ($n = 12$), 6.3% ($n = 8$), 8.6% ($n = 4$) and 7.6% ($n = 3$) at 3, 6, 12 and 24 months, respectively.

533. Abnormal pancreatic exocrine function in obesity, a possible consequence of insulin resistance

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Pancreatic amylase content and amylase mRNA levels are insulin-dependent, being markedly reduced in insulinopenic states. Thus, the exocrine pancreas is an insulin-sensitive tissue. Since insulin resistance occurs in other tissues in obesity, the purpose of the study was to characterize the abnormalities of pancreatic exocrine function occurring in this state using the adult obese Zucker rat. It was found that the amylase content of obese pancreases was reduced by 30–40% compared to lean controls. Reduction in content was not due to increased secretion, since this was similar under basal conditions and reduced by between 20 and 40% in isolated acini of obese rats during stimulation with the cholecystokinin analogue, caerulein (10^{-11}–10^{-8} mol/l). The mRNA levels for pancreatic amylase were approximately 25% lower in obese than in lean pancreases. Glucose utilization, measured as $^{14}CO_2$ production from [U-^{14}C]glucose, was reduced by 60% under basal conditions and by > 70% in the presence of insulin (0.15–0.15×10^6 mU/l) in isolated acini of obese rats compared to lean controls. In conclusion, pancreatic exocrine function is disturbed in the obese Zucker rat; the findings are consistent with a state of insulin resistance in this tissue.

534. Collagen binding activity in sera of patients with Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes

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Accumulation of altered vascular collagen may be implicated in the initiation of an autoimmune response leading to the vascular complications of diabetes. The aim of this study was to investigate the presence of collagen binding capacity (CBA) in diabetic sera. A solid-phase was coated with type I, III, IV collagen and consecutively incubated with serum dilutions and alkaline-phosphatase coupled anti-human IgG. Blocking assays were carried out by pre-incubation of sera with collagen. Non-specific binding of immune complexes (CIC) was investigated by testing sera for CIC (anti-C3 EIA) and by 2–4% PEG precipitation of sera before CBA detection. CBA (for at least one type of collagen) was found in 49% of 35 patients with Type 1 diabetes, in 43% of 23 patients with Type 2 diabetes but only in 10% of 30 normal sera. CIC were detected in 56% of sera with CBA. CBA, however, was inhibited by PEG precipitation in only a few cases (20%). CBA did not correlate with age, duration of diabetes or glucose control. The highest levels were found to be associated with severe diabetic complications. Although the pathogenesis of diabetic complications is multifactorial, these data further implicate the immune system.

535. Cephalic phase modulates hepatic insulin extraction and glucose tolerance in normal man

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We studied the effect of cephalic stimulation (sham-feeding) on insulin secretion, insulin hepatic extraction and glucose tolerance. Blood glucose, plasma C-peptide and insulin (IRI) were measured in seven normal subjects during intravenous administration of 25 g glucose with and without sham-feeding during the initial 10 min. The amount of insulin secreted was the same with and without sham-feeding, as evaluated by the area under the C-peptide curves (94 ± 8 versus 102 ± 8 mmol/l × 180 min, $p > 0.05$). Also the total areas under the IRI curves were similar 1821 ± 288 versus 2034 ± 352 mU/l × 180 min; $p > 0.05$. In contrast to the superimposable C-peptide curves, the IRI profiles differed during the first 50 min after intravenous glucose with sham-feeding, resulting in a 24% greater IRI area and a significantly lower C-peptide ($p < 0.05$): IRI ratio suggesting a decreased hepatic insulin extraction. The blood glucose concentrations were also significantly lower after intravenous glucose with sham-feeding ($p < 0.03$), the incremental area was 64% of that seen without sham-feeding. In conclusion, the cephalic phase of sham-feeding has no effect on insulin secretion, but alters hepatic insulin extraction and improves glucose tolerance.

536. Metabolic and obstetrical results in Type 1 (insulin-dependent) diabetic pregnancy: pump versus optimized conventional insulin therapy

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Strict blood glucose control during diabetic pregnancy is able to prevent feto-maternal complications. We compared the efficacy of continuous subcutaneous insulin infusion (CSII) and optimized conventional treatment (OCT) (three daily injections) in 12 Type 1 diabetic, normal weight women (aged 27.5 ± 1.2 years) randomly assigned to CSII (six patients) or OCT (six patients) before week 10 of pregnancy. We performed weekly: blood glucose profiles (07.00, 09.00, 12.00, 15.00, 19.00, 22.00, 03.00 h), plasma oestradiol and human placental lactogen (from week 21); monthly: HbA_1 and conceptus ultrasound evaluation. Insulin requirement ($U \cdot kg^{-1} \cdot day^{-1}$) was comparable in the two groups. Blood glucose values of CSII were not significantly different (t-test) from OCT (mean ± SEM): 5.0 ± 0.7 versus 5.5 ± 0.7 mmol/l in the first trimester; 5.3 ± 0.6 versus 5.6 ± 0.8 mmol/l in the second trimester; 5.4 ± 0.6 versus 5.4 ± 0.8 mmol/l in the third trimester. HbA_1 values were similar in CSII and OCT. Oestradiol and human placental lactogen values and ultrasound evaluations were all normal. Pregnancy weight gain was similar in the two groups: 9.0 ± 3.3 versus 10.1 ± 0.9 kg. Delivery was always at term and fetal weights were appropriate for gestational age. Neither perinatal mortality nor respiratory distress syndrome were seen. These data suggest that CSII offers no significant advantage over OCT in the management of Type 1 diabetic pregnancy.

537. Influence of insulin-induced hypoglycaemia on platelet sensitivity to a platelet activating factor

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In insulin-treated diabetic patients, a close temporal relationship has been reported between sudden microvascular accidents and hypoglycaemia. Among the possible mechanisms involved in this phenomenon, we studied the influence of insulin-induced hypoglycaemia on platelet sensitivity to a powerful physiological aggregating agent, the platelet activating factor: 1-0-alkyl-2-acetyl-sn-glycero-3-phosphocoline (AGEPC). Five healthy male subjects (mean age 26 ± 1 years) received, after overnight fast, a 60-min intravenous insulin infusion (64 mU/m^2 per min) with serial measurements of plasma glucose and of counter-regulatory hormones. Before infusion (0 min), at hypoglycaemic nadir (42 ± 3 min), at 90 and 150 min the platelet aggregation induced in vitro by AGEPC (7.5×10^{-8} mol/l–1.7×10^{-7} mol/l) was determined. Plasma glucose (mmol/l) was (mean ± SEM): 5.3 ± 0.2 at 0 min; 2.8 ± 0.4 at nadir; 4.1 ± 0.7 at 90 min; 5.3 ± 0.4 at 150 min. Maximal aggregation, measured as OD at T_0–minimum OD/OD at T_0, was (mean ± SEM): 30 ± 4% at 0 min; 48 ± 5% at nadir ($p < 0.05$ versus 0 min); 18 ± 5% at 90 min ($p < 0.005$ versus 0 min); 18 ± 4% at 150 min ($p < 0.02$ versus 0 min). In the four subjects who showed a reversible initial aggregation curve, the curve became irreversible at nadir and again reversible in the recovery phase. This study demonstrates that insulin-induced hypoglycaemia deeply influences platelet sensitivity to AGEPC, with an initial increase and a decrease in the recovery phase. Data are interpreted as a metabolic and hormonal modulation on platelet sensitivity to AGEPC.

538. Pump accuracy of miniaturized insulin infusion systems

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Reliability and precision of medical devices have to be proved. Therefore, first and second generation insulin pumps (Siemens Promedos E 1, Mill Hill Infuser, Autosyringe 6MP and Nordisk Infuser) were tested under defined conditions (n = 5 each). The output of insulin was electronically measured every 15 min over a 10-h period. When possible, low (LR, 10 µl/h) and high (HR, 20 µl/h) pump rates were tested. Remarkable deviations from the prescribed values were found. Promedos mean 1-h pump rates deviated from −3% to 2.3% (HR) and −5% to 1.8% (LR). All Mill Hill infusers showed too low (−9.5% to −11.4%) mean 1-h pump rates as did Autosyringe 6MP: −2.1% to −4.4% (HR) and −3.1% to −10.2% (LR). Deviation of Nordisk mean 1-h pump rates was −3.7% to 9.6% (HR), but constantly negative in low rate mode (−3.3% to −6.3%). The biggest maximal pump rate deviation from hour-to-hour was observed for Nordisk Infuser (39% of mean pump rate, HR), whereas smallest maximal hour-to-hour variance was observed for Promedos E 1 (9%, HR). From these results, systematic technical check-up is suggested for insulin infusion pumps. Improvement of handling comfort is not correlated to an improvement in precision. Accuracy of insulin delivery may depend much more on the many problems of insulin infusion systems than from the guaranteed electronical precision of the pump motor.

539. Lack of benefit from acute elevation of plasma glibenclamide levels before meals

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The hypoglycaemic action of glibenclamide is prolonged by slow tablet dissolution. Glibenclamide solid solution (GSS, Hoechst) is a new formulation in which the active drug is more rapidly absorbed. Twelve non-insulin-dependent diabetic patients (nine males, three females, mean ± SD age 58 ± 6.6 years, mean ± SD duration of diabetes 4.3 ± 6.1 years) consented to a randomised cross-over study taking conventional glibenclamide (mean dose 7.5 mg twice daily) or GSS (three times daily) in equivalent doses 30 min before meals. Standardised inpatient 24 h metabolic profiles were performed after 1 week on each therapy. No differences were observed between therapies in mean plasma glucose or geometric mean plasma insulin profiles in spite of higher plasma glibenclamide levels on GSS (118.5 ± 92.3 versus 35.7 ± 29.0 ng/ml before breakfast). A third 24 h profile, with double doses of GSS for 1 day only, gave plasma glibenclamide levels of 302.6 ± 168.2 ng/ml before breakfast but with no improvement in glucose or insulin profiles. Hypoglycaemia was not more frequent on any one treatment. In conclusion, acute elevation of plasma glibenclamide levels before meals, in patients on glibenclamide therapy, appears to have no additional beneficial effect.

540. Adrenaline inhibition of insulin release is accompanied by a paradoxical rise in cytosolic Ca^{2+} in RINm5F cells

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It has been suggested that adrenaline inhibits insulin release by lowering cytosolic Ca^{2+} ($[Ca^{2+}]_i$). We have measured changes in insulin release and $[Ca^{2+}]_i$ in parallel using RINm5F cells loaded with the fluorescent Ca^{2+}-indicator Quin 2. Alanine (10 mmol/l), which depolarizes the cells and raises $[Ca^{2+}]_i$, stimulated insulin release threefold (10 min). Adrenaline (1 µmol/l) inhibited alanine-induced insulin release by 65%, while the α_2-adrenergic agonist clonidine (10 µmol/l) caused a 55% decrease. The α_1-agonist phenylephrine (10 µmol/l) only inhibited insulin release marginally. Basal insulin release was not altered by the agonists. Adrenaline rapidly and transiently increased $[Ca^{2+}]_i$ under basal and alanine-stimulated conditions. Phenylephrine also increased $[Ca^{2+}]_i$ whereas clonidine did not. The selective α_1-antagonist BE 2254 abolished the adrenaline-induced rise in $[Ca^{2+}]_i$. Adrenaline is able to mobilize calcium, since the rise in $[Ca^{2+}]_i$ was still present in Ca^{2+}-free medium, where the effect of alanine is abolished. In conclusion, adrenaline activates both α_1- and α_2-adrenergic receptors on RINm5F cells. As in hepatocytes a rise in $[Ca^{2+}]_i$ is mediated via α_1-receptors. It is suggested that the α_2-adrenergic inhibition of insulin release is not related to changes in $[Ca^{2+}]_i$ but rather is exerted at a more distal step in the secretory mechanism.

541. Five patient years with the Pen Infuser

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The system comprises a 3 ml disposable syringe and a 24 inch catheter combined with a subcutaneous needle. The system is filled with U-100 regular insulin (Actrapid) and the infuser is operated manually. Bolus doses are given seven times daily from 07.00 to 22.00 h. To achieve an acceptable overnight metabolic control, an extra injection of medium acting insulin (Semilente) is given subcutaneously every night. The patient material comprises eight insulin-treated C-peptide-negative diabetic patients in unacceptable metabolic control on conventional insulin therapy with two to five daily injections. During an observation period of about 5–9 months the mean HbA_{1c} decreased from 11.0 ± 2.1 to 8.8 ± 2.0% (upper reference limit 7.0%). The mean blood glucose value during the observation period was 7.2 ± 1.3 mmol/l with 36% of the values above 8.0 mmol/l and 14% below 3.5 mmol/l. The total daily insulin dose was reduced in most subjects from mean 0.63 ± 0.1 to 0.54 ± 0.1 IU/kg. The mean body weight did not change. The anti-atherogenic index of HDL- and LDL-cholesterol increased in all subjects. There were no severe complications during the observation time. Patient opinions were very positive and all patients wished to continue with the Pen Infuser at the end of the study.

542. Radioimmunoassay for glucagon-like-peptide-1: presence in human pancreas and glucagonomas

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Mammalian glucagon mRNA and the human pre-proglucagon gene encode two further glucagon-like peptides, GLP-1 and GLP-2. The amino-acid sequence of GLP-1 is completely conserved in three mammalian species and has only slightly less homology with the equivalent anglerfish peptide than has glucagon. It thus has the appearance of a highly conserved hormonal peptide, but whether post-translational processing produces GLP-1 or other fragments is unknown. We have developed a radioimmunoassay to synthetic GLP-1-(1-19)-peptide. Rabbits immunised with a bis-diazobenzidine bovine serum albumin conjugate yielded antisera cross-reacting with synthetic GLP-1 whole molecule. The selected antiserum cross-reacts 20% with GLP-1 and has a detection limit of 50 pmol/l (whole molecule), but does not cross-react with glucagon or other pancreatic peptides. Chromatography of human pancreas extracts on Sephadex G-50 gave peaks of N-terminal GLP-1-like immunoreactivity at Kav 0.10, 0.36, 0.56 (the position of synthetic GLP-1) and 0.64. An extract of glucagonoma contained a preponderance of larger molecular forms, and plasma from a glucagonoma patient gave peaks of 69 nmol/l at Kav 0.22, and 2.3 nmol/l at Kav 0.56. The results suggest that post-translational processing yields several larger and one smaller molecular form, in addition to GLP-1 as defined by its delimiting pairs of basic amino-acid residues.

543. Cardiovascular risk factors in relation to coronary heart disease in newly diagnosed Type 2 (non-insulin-dependent) diabetic patients and normal subjects

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The prevalence of coronary heart disease (CHD) and the relationship of CHD to cardiovascular risk factors were assessed in 133 newly diagnosed Type 2 diabetic patients (70 men, 63 women), aged 45–64 years, and in 144 randomly selected non-diabetic control subjects of the same age group (62 men, 82 women). The age-adjusted prevalence of CHD (defined by symptoms and ECG abnormalities suggestive of CHD) was 3.5-fold in male ($p = 0.001$) and 3.1 ($p = 0.001$) in female diabetics compared with the corresponding non-diabetic subjects. There was no significant difference in serum total cholesterol level between diabetic and non-diabetic subjects, but HDL-cholesterol was lower and triglyceride level was higher in the diabetic compared with that in non-diabetic subjects. The prevalence of hypertension (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 95 mmHg or on drug treatment for hypertension) and obesity was increased in diabetic compared with non-diabetic subjects. Fasting serum insulin level was higher in diabetic patients, but no difference was found in 2-h post-glucose serum insulin level between diabetic and non-diabetic subjects. In multiple logistic analyses (including age, history of smoking, presence or absence of hypertension, cholesterol, triglycerides, HDL-cholesterol, 2-h post-glucose serum insulin, body mass index and diabetes +/−) carried out separately in men and women, age, hypertension and diabetes showed an independent association with CHD in men, whereas in women only smoking, high 2-h post-glucose serum insulin and diabetes did so.

544. Isotopic capillary permeability test and long-term complications of diabetes

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An elevated transcapillary escape rate of albumin has been reported in diabetes. Evidence of increased capillary permeability has been found with a non-invasive isotopic test, in cases of idiopathic oedema. After injection of ^{99m}Tc-albumin IV, radioactivity disappearance was measured externally after removal of forearm venous compression. We used this test on 30 Type 1 (insulin-dependent) and 40 Type 2 (non-insulin-dependent) diabetic patients (aged 50.1 ± 1.9 years; mean ± SEM; duration of diabetes 10.4 ± 1.0 years) and on 25 normal volunteers. Albumin retention at 10 min was significantly elevated in 11 patients. The radioactivity disappearance curve was analyzed using fast-function transfer (Fourier analysis). The amplitudes found were analyzed by comparing low and high frequency (LF and HF). The HF/LF amplitudes were above 1% in these 11 patients and also in 60% of the 59 other diabetic subjects, whereas it was below 1% in all 25 controls, showing the high incidence of increased capillary permeability in diabetics. These abnormal results correlated with retinopathy and/or nephropathy, but not with the duration or type of diabetes. We thus conclude that albumin wash-out takes both the venous and lymphatic routes and that plasma constituent extravasation is related to the thickening of the capillary basement membrane.

545. Liver glycogen metabolism in starved-refed lean and genetically obese *(fa/fa)* rats

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Genetically obese rats are characterized by hyperinsulinaemia, and post-prandial hyperglycaemia, together with elevated portal blood lactate and pyruvate concentrations. Despite this situation which should be favourable to enhance hepatic glycogen synthesis, there was no difference in the accumulation of glycogen in livers of 24-h starved lean or obese animals 2, 6 and 10 h after a standard meal. The glycogen synthase *a* levels were the same in both groups and did not increase after feeding; phosphorylase was inactivated upon feeding only in livers of lean rats, whereas [glucose 6-P] and [fructose 6-P] increased in both groups, indicating that glycogen synthesis was driven by substrate availability, i.e. by a 'push mechanism'. The lack of phosphorylase inactivation in livers of refed obese rats might, by maintaining glycogenolysis, counteract a potential increase in glycogen accumulation. The impaired hepatic phosphorylase inactivation of obese animals could be due to insulin resistance. As glucose also increases after a meal and is known to favour phosphorylase inactivation, a defective glucose effect on the enzyme cannot be ruled out. Another explanation would be a sustained trigger on phosphorylase activation, counteracting the expected inactivation: such a situation was suggested by this laboratory for hepatocytes from fed genetically obese (ob/ob) mice.

546. The effect of improved control on serum and erythrocyte fatty acid composition in Type 1 (insulin-dependent) diabetic patients

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The effect of continuous subcutaneous insulin infusion on serum and erythrocyte lipids was studied in seven previously poorly-controlled Type 1 diabetic patients, aged 23–36 years. The mean blood glucose during a 24-h profile fell from 11.3 to 8.3 mmol/l and HbA_1 from 10.1 to 7.5% (normal $5.9 \pm 0.5\%$, mean ± SD). In serum, total cholesterol and triglycerides decreased ($p \leq 0.02$ and $p \leq 0.05$, respectively) and the relative amounts of oleate and total monounsaturated fatty acids (MUFA) decreased ($p \leq 0.05$ for both), whereas linoleate, arachidonate, lignocerate and total polyunsaturated fatty acids (PUFA) increased ($p \leq 0.05$, $p \leq 0.02$, $p \leq 0.02$ and $p \leq 0.05$, respectively). In red cells, the relative amounts of total saturated fatty acids, oleate and MUFA decreased ($p \leq 0.05$ for all) whereas arachidonate and PUFA increased ($p \leq 0.02$ for both). In addition, the red cell arachidonate: linoleate ratio increased ($p \leq 0.02$). In conclusion, improved control changed serum and red-cell fatty acid composition.

547. Down-regulation of insulin receptors in primary cultured cardiac myocytes

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Information concerning cardiac insulin receptor regulation is lacking. Primary cultured cardiocytes from adult rats were used here to approach this problem. These cells exhibited specific binding of ^{125}I-insulin which was reversible, time- and temperature-dependent, and constant over 3 days. Curvilinear Scatchard plots of insulin binding were observed during this period. K_d calculated for the high-affinity segment was 4.5×10^{-10} mol/l and identical to the value observed in freshly-isolated cardiocytes. Exposure of cardiocytes to high concentrations of insulin (1.7×10^{-7} mol/l) for different periods (2–60 h) resulted in increasing down-regulation of the insulin receptor, reaching a maximal decrease in insulin binding by 45% after 48–60 h. Scatchard analysis of insulin binding to down-regulated cells suggested a reduction in the number of binding sites. Cycloheximide did not affect down-regulation after 6 h in spite of total inhibition of protein synthesis, but increased down-regulation after 24 h by 35%. After removal of insulin from the culture medium down-regulation was reversible, whereas cycloheximide inhibited this process. In conclusion, our data show that insulin can inversely regulate its own receptor in the heart. This process appears to be due to a reduction in the number of binding sites and to be independent of protein synthesis.

548. Identification of functional insulin receptors on membranes from an insulin producing cell line (RINm5F)

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Although pancreatic β cells can bind insulin, their insulin receptors have not been characterized and the impact of insulin binding on β cell function is unclear. To overcome the limited amount of tissue available using isolated islets, we studied insulin receptors on membranes obtained from RINm5F cells, a cloned, insulin-producing, rat pancreatic cell line. To study the insulin receptor α-subunit, ^{125}I-photoreactive insulin was covalently bound to the membranes in the absence or presence of unlabelled insulin. SDS-polyacrylamide gel electrophoresis under reducing conditions revealed the specific labelling of a unique protein with M_r 130,000, corresponding to the α-subunit. The putative insulin receptor β-subunit was studied using a cell-free phosphorylation assay. Analysis of the labelled proteins showed that RINm5F cell membranes contain a phosphoprotein with M_r 95,000, whose level of phosphorylation was selectively increased by insulin. Moreover, this phosphoprotein was specifically immunoprecipitated by antibodies to insulin receptors. The insulin receptors of RINm5F cells (and, by analogy, possibly of native β cells) appear similar to those of previously studied insulin target cells. The insulin-stimulated protein kinase of the receptor β-subunit could thus play a role in regulating RINm5F cell metabolism and/or insulin release.

549. Lack of high affinity insulin receptors on purified pancreatic A cells

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It has been suggested often that glucose regulation of glucagon release is an insulin-dependent process. To test this hypothesis, experiments were undertaken to identify insulin receptors on pancreatic A cells, which were purified by autofluorescence-activated cell sorting. A sensitive insulin receptor assay was developed, employing ^{125}I- or ^{123}I-labelled hormone. For both tracers, monoiodo Tyr-A14 insulin was obtained by reverse phase high performance liquid chromatography. Binding studies on IM-9 lymphocytes indicated the validity of both tracers. With ^{125}I-insulin, specific binding on IM-9 lymphocytes was still detectable at 5.10^4 cells/ml; in contrast, no specific binding was measured on purified A cells at 5.10^5 cells/ml. Due to its higher specific activity, ^{123}I-insulin increased the sensitivity of the binding assay to IM-9 lymphocytes fivefold. However, using this tracer, specific binding on A cells remained undetected. In conclusion, pancreatic A cells contain < 400 high affinity ($K_a \sim 10^9$ l/mol) insulin receptors per cell, which is 50–500-fold lower than in classical insulin target cells. These findings are in good agreement with the absence of insulin effects upon glucose transport in purified A cells. On the basis of these observations, revision of earlier concepts concerning the role of insulin in glucagon release might be required.

550. Effects of leucine on islet palmitate metabolism and insulin release by isolated islets of fed and starved rats

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Glucose (20 mmol/l) inhibition of palmitate oxidation and stimulation of its incorporation into islet lipids are blocked after 48 h starvation. We have investigated whether similar changes occur with leucine. (^{14}C)-Palmitate (0.5 mmol/l) oxidation was measured as the production of $^{14}CO_2$ and (^{14}C)-acetoacetate. Its incorporation into islet phospholipids (PL) and triacylglycerols (TG) was quantified after lipid extraction and thin-layer chromatography. Insulin release was studied in static incubations. Leucine (10 mmol/l) induced a twofold stimulation of insulin secretion and almost abolished the oxidation of palmitate without modifying its incorporation into TG and PL. Starvation (48 h) did not induce any significant effect. Addition of glucose (3 mmol/l) provoked a tenfold increase of insulin release and significantly increased the labelling of TG and PL without altering leucine inhibition of palmitate oxidation. Starved islets showed a higher (100%) rate of palmitate oxidation at 3 mmol/l glucose. Addition of leucine (10 mmol/l) almost abolished the oxidation of palmitate and stimulated its incorporation into TG and PL as well as insulin release to values identical to those obtained with fed islets. Starvation does not block leucine inhibition of islet palmitate oxidation. This allows an increased rate of palmitate esterification (TG and PL) at 3 mmol/l glucose and a potentiation of insulin release.

551. Distribution of an Apo A-I-C-III DNA polymorphism in different racial groups

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A restriction fragment length polymorphism has been identified within the Apo AI-CIII gene cluster which segregates with types IV and V hypertriglyceridaemia in Caucasoid individuals. This DNA polymorphism is situated at the 3′ end of the Apo-CIII gene which lies approximately 2.6 Kb downstream of the Apo-AI gene on chromosome 11. Using the restriction endonuclease Sst-I, Southern blot-hybridization techniques and a ^{32}P Apo-AI gene probe, 30 out of 74 Caucasoid hypertriglyceridaemic patients (frequency = 0.22) were found to possess an uncommon 3.2 Kb band detected on autoradiography. The frequency in 52 normolipidaemic Caucasoids was only 0.05. To investigate the prevalence of this polymorphic allele in non-Caucasoid normolipidaemic individuals, 89 subjects of different racial origins were genotyped. The allele frequency observed was: Chinese: 0.475 ($n = 20$); Japanese: 0.19 ($n = 21$); Asians: 0.18 ($n = 28$); and Africans: 0.15 ($n = 20$). The prevalence of the polymorphism is thus increased in non-Caucasoid individuals and there appears to be no disease association in these groups. However, there may be heterogeneity of the Sst-I restriction sites at this locus, i. e. the sites in non-Caucasoid and hypertriglyceridaemic subjects may be different but within 100 bp of each other.

552. Similar metabolic effects of pulsatile versus continuous intravenous human insulin delivery during euglycaemic hyperinsulinaemic glucose-clamp in normal man

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Seven normal volunteers were studied on two different occasions when 4 h pulsatile ($0.8\ mU \cdot kg^{-1} \cdot min^{-1}$, 7.5 min out of 15) and continuous ($0.4\ mU \cdot kg^{-1} \cdot min^{-1}$) intravenous infusions of human insulin (Actrapid HM, Novo) were randomly compared. A euglycaemic glucose-clamp was used and a 3-^{3}H-glucose infusion was utilized for determination of endogenous glucose production and metabolic clearance rate (MCR) of glucose. Plasma glucose was similar in both conditions; plasma insulin was stable at about 28 mU/l (during continuous infusion) and fluctuated between 10 and 45 mU/l (mean: 29 mU/l) in the pulsatile infusion. Exogenous glucose infused was 1.137 ± 0.058 and $1.088 \pm 0.099\ g \cdot kg^{-1} \cdot 4h^{-1}$ in both infusion groups, respectively (NS). Endogenous glucose clamp was totally suppressed in both conditions. Glucose MCR increased similarly to a maximum of 6.71 ± 0.19 (continuous infusion) and 6.79 ± 0.59 (pulsatile infusion) $ml \cdot kg^{-1} \cdot min^{-1}$ during the fourth hour. Plasma C-peptide levels remained stable whereas plasma glucagon, non-esterified fatty acids and β-hydroxybutyrate were similarly suppressed in both tests. Thus, in these conditions, pulsatile and continuous insulin infusions have similar metabolic effects. These data contrast with those of Matthews et al. (1983) who reported that, at lower plasma concentrations (5–19 mU/l), pulsatile insulin had a greater hypoglycaemic effect than continuous delivery.

553. Activated T-lymphocytes in insulin-dependent diabetes: evidence for an environmental effect

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Expression of HLA-DR antigens on T-lymphocytes indicates that these cells are actively involved in an immune response. Recently diagnosed Type 1 (insulin-dependent) diabetic patients show increased levels of activated T-cells and, although the level declines over the next 5 years, it remains above normal. To discover whether expression of these antigens is determined by genetic factors, we studied lymphocytes from identical twins discordant for Type 1 diabetes, using a monoclonal anti-Ia antibody. Of seven twin pairs discordant for Type 1 diabetes for < 5 years, significantly increased levels of activated T-cells were found in both the diabetic and non-diabetic twin (8.6 ± 4.2 and $7.9 \pm 2.6\%$, respectively) compared with healthy control subjects ($2.2 \pm 0.23\%$). In six twin pairs discordant for Type 1 diabetes for > 5 years, the levels in the diabetic and the non-diabetic twins (4.8 ± 2.2 and $3.4 \pm 2.2\%$, respectively) were lower than in the short-term discordant twins. In both short- and long-term discordant pairs,

the diabetic had significantly higher levels of activated T-lymphocytes than the non-diabetic co-twin. As differences between identical twins must be due to non-genetic factors, we conclude that activation of T-lymphocytes is determined, at least in part, by non-genetic factors. Most unaffected co-twins of recently diagnosed Type 1 diabetes have probably been exposed to this environmental stimulus.

554. Discrepancy between insulin-dependent and non-insulin-dependent diabetes in the interrelationship between red-blood-cell sorbitol and glycaemia

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The red-blood-cell sorbitol content (RBC-SOR) is considered a useful marker of the activity of the sorbitol pathway which could be involved in the genesis of some diabetic complications. In this study RBC-SOR was measured with a fluorimetric enzymatic assay and expressed as nmol/g haemoglobin. RBC-SOR was significantly higher in a group of 114 diabetic patients than in a matched control group (mean RBC-SOR 139.4 ± 90.0 versus 40.6 ± 8.8 nmol/g, $p < 0.001$). In 58 insulin-dependent diabetic patients a significant linear correlation was found between blood glucose and RBC-SOR ($r = 0.33$, $p < 0.02$). However, unusually high RBC-SOR was linked to low blood glucose values in 8.6% and vice-versa in 16%. Since changes of blood glucose are paralleled ($p < 0.01$) by equal changes in RBC-SOR during the acute treatment of newly-diagnosed patients, there must exist individual differences in polyol pathway activity depending on unknown factors. Moreover, no significant correlation could be detected between glycaemia and RBC-SOR in 56 non-insulin-dependent diabetic patients despite the absence of any significant difference in these variables between the two populations. As a whole RBC-SOR does not appear to be related to the age or sex of the patients, or to the duration or degree of control (HbA_1) of the diabetes.

555. Cold reacting lymphocytotoxin in recent-onset Type 1 (insulin-dependent) diabetes

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Antibodies directed against lymphocytes have been described in various autoimmune diseases and Type 1 diabetes. To see whether this immune abnormality is related to the pathogenesis of diabetes, we looked for non-HLA cold reacting lymphocytotoxins (CRL) in Type 1 diabetic patients of various duration and correlated the presence of such antibodies to cytoplasmic islet cell antibodies (ICA) and HLA-A, -B, -DR antigens. Lymphocytotoxins were determined on 21 frozen-thawed lymphocyte suspensions of an unrelated blood donor's panel typed of HLA-A, -B, -C, -DR, -ST and -MT antigens, by complement dependent cytotoxicity at 20 °C and 4 °C. 19 recent onset Type 1 diabetic patients (< 5 months), 13 Type 1 diabetic patients with a duration ranging 6–24 months, 10 long standing Type 1 diabetic patients (> 9 years) and 10 non-diabetic control subjects were studied. Polytransfused subjects and multiple pregnancy were excluded. Seven patients among the recent-onset diabetics exhibited CRL (37%). This frequency fell to 15% after 6 months. In long-standing diabetic patients and controls, frequency of CRL did not exceed 10%. CRL were not associated with conventional ICA. There was a tendency for an association with HLA-DR3 but not with DR4. Temperature conditions and intensity of lysis suggest that the immunoglobulins are IgM and recognize both B and T lymphocytes.

556. Evaluation of diabetic retinopathy in a group of 56 Type 1 (insulin-dependent) diabetic patients treated with continuous insulin infusion

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Retinal changes were evaluated ophthalmoscopically and by fluoroangiography in 56 Type 1 diabetic patients during continuous insulin infusion by pump (51 subcutaneous and 5 intraperitoneal) during a total of 1160 patient-months of treatment (20 ± 12 months/patient mean ± SD). The mean age of the patients was 33 ± 12 years, mean duration of diabetes 12 ± 7 years. Steady good control was achieved on continuous insulin infusion: initially HbA_1 $10.9 \pm 2.2\%$ and mean blood glucose 12.62 ± 5.27 mmol/l, after 3 month $8.67 \pm 1.9\%$ and 7.62 ± 3.41 mmol/l, and after one year $8.33 \pm 1.62\%$ and 7.42 ± 3.25 mmol/l, respectively. After exclusion of eyes with previous laser treatment (12) or opacities (15), 85 eyes were evaluated. Initially 59 eyes showed no retinopathy, while 26 had background retinopathy. After 3 and 6 months of treatment, there was deterioration in 2% and 6% respectively of the retinopathy-free eyes, and 12% and 14% of those with retinopathy. After a period of one year the retinopathy-free eyes had worsened in 9.3% and those already affected in 50%; these percentages became respectively 20% and 25% after a period of 2 years. In 25 eyes with a follow-up longer than 24 months deterioration was noted in 10.5% and 33%.

557. β-cell secretion in non-insulin-dependent diabetes of young people

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Type 2 (non-insulin-dependent) diabetes is a heterogeneous disorder whose basic defect has not yet been established. An interplay between β-cell inadequacy and insulin resistance in Type 2 diabetes has been postulated. Moreover, insulin secretion patterns of young people with Type 2 diabetes are not well defined. In this study, β-cell secretion of six Type 2 diabetic patients (mean aged 27 ± 4 years, ideal body weight $107 \pm 8\%$, two females and four males) was studied. Diabetes controlled without insulin (at least 5 years), was diagnosed before age 25 years. β-cell secretion was evaluated by an oral glucose tolerance test (75 g), an intravenous glucose tolerance test (0.3 g/kg) and an arginine test (5 g intravenously). Seven days of unrestricted diet and oral hypoglycaemic withdrawl preceded the tests. Six healthy subjects matched for sex and age served as controls. The results showed: near-normal basal insulin levels (13.3 ± 1.2; controls: 14.2 ± 1.4 mU/l); low insulin response after oral glucose (mean IRI increments 0–120 min: 8.1 ± 3.6; controls: 36.1 ± 6.2 mU/l); markedly impaired first phase insulin secretion after intravenous glucose (IRI area 0–10 min: 17.6 ± 10.2; controls: 613.6 ± 95.4 mU/l); near-normal insulin response to arginine (IRI area 0–10 min: 140.2 ± 31.2; controls: 107.2 ± 32.3 mU/l). From these data, young people with non-insulin-dependent diabetes seem to present a near-normal β-cell response to non-glucose stimuli, a pattern described previously for late onset Type 2 diabetes.

558. Hypertriglyceridaemia and peripheral vascular disease in Type 1 (insulin-dependent) diabetes

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Hypertriglyceridaemia (HTG) is the most frequent lipid disorder in diabetes subjects with peripheral vascular disease (PVD). In spite of increasing knowledge, the arteriosclerotic risk of HTG is still controversial. It is of interest to know whether triglyceride-rich lipoprotein may be a better predictor of the risk of atherosclerosis than total triglycerides. Therefore 77 Type 1 diabetic patients (23 males, 54 females, aged 52 ± 18 years, $119 \pm 21\%$ Broca) with HTG (triglyceride ≥ 2.26 mmol/l) and 97 Type 1 diabetic patients (39 males, 68 females, aged 47 ± 18 years, $116 \pm 22\%$ Broca) without HTG (triglyceride < 2.26 mmol/l, cholesterol < 6.72 mmol/l) were studied. Lipoprotein analyses were performed according to the LRC-program and lipids were measured with commercial kits. The diagnosis of PVD was made clinically and by oscillography. Clinical data show that PVD was present in 45% of patients with HTG and in 32% of patients without HTG. In all patients HDL contained less cholesterol than controls ($p < 0.05$ with HTG; $p < 0.005$ without HTG) and VLDL contained more cholesterol in PVD than in controls ($p < 0.025$). A negative relationship between VLDL-cholesterol and HDL-cholesterol was only significant in diabetic patients without HTG ($p < 0.001$). Taking a ratio of VLDL/HDL-cholesterol of ≥ 1.0, 65% of all PVD were selected, while a ratio of < 1.0 selected 61% of all controls. It is concluded that the ratio of VLDL/HDL-cholesterol is a better indicator for the risk of arteriosclerosis in HTG than the measurement of total triglycerides levels.

559. Importance of substrate competition in the mechanism of insulin resistance in man

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Carbohydrate (CHO) oxidation induced by a glucose or a fructose ($0.5\ g \cdot kg^{-1} \cdot h^{-1}$) infusion over 2 h was compared by means of continuous indirect calorimetry in six normal subjects with or without a concomitant intralipid infusion. Glucose infusion was accompanied by a rise over basal values in both plasma glucose (5.55 ± 0.66 mmol/l) and insulin (29.7 ± 7.5 mU/l) levels, with a further rise of both curves during Intralipid infusion (7.67 ± 0.44 mmol/l and 43.4 ± 11.0 mU/l). By contrast, plasma glucose and insulin rose only minimally during fructose infusion (0.18 ± 0.19 mmol/l and 4.0 ± 1.6 mU/l, respectively

without Intralipid and 0.62 ± 0.17 mmol/l and 7.5 ± 2.3 mU/l with Intralipid). During the 2 h sugar infusion, total CHO oxidation was 2.1 ± 0.1 mg·kg^{-1}·min^{-1} for glucose and 3.4 ± 0.3 mg·kg^{-1}·min^{-1} for fructose. A significant fall was observed during Intralipid both for glucose (1.5 ± 0.2 mg·kg^{-1}·min^{-1}, $p < 0.005$) and fructose (2.5 ± 0.3 mg·kg^{-1}·min^{-1}, $p < 0.01$). Lipid oxidation was increased in both cases during Intralipid infusion. These observations show that the lipid-induced inhibition of CHO oxidation is observed both with glucose and fructose infusion, suggesting that insulin is not primarily involved. These results support a metabolic origin for insulin resistance.

560. The influence of diabetes mellitus on lens transparency

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Diabetes mellitus predisposes to cataract. However, until recently no simple and reliable methods were available to assess loss of light transmission by the lens as a stage of cataract development. We developed a computer-fluorophotometric method to measure transmission of blue-green light by the lens, by comparing the natural peal fluorescence values of the anterior and posterior part of the lens. Reproducibility is within 3%. Fifty-two healthy subjects and 67 diabetic patients (duration of diabetes 1–48 years) without cataract and aged between 18 and 84 years were studied. In the control group of healthy subjects the lens transmission amounted to about 100% up to the age of 50 years and was followed by an exponential decline. In the group of diabetic patients this decline occurred on average 15 years earlier. In diabetic patients the age-corrected lens transparency showed an extra loss of 5% for every 10 years of diabetes. Variation among diabetic patients of the same age is three times as great as that in the healthy controls. This may reflect different levels of diabetic control since diagnosis. Computer fluorophotometric measurement of lens transmission may offer a new opportunity to assess an early stage of cataract formation in diabetic patients and to evaluate factors that might influence this process.

561. Insulin effects on protein phosphorylations in sarcolemmal membranes mediated by GTP-regulatory proteins

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This work is concerned with membrane protein phosphorylation as the initial events in insulin action. Isolated sarcolemmal membranes were incubated with ^{32}P-γ-ATP and the proteins separated by SDS-PAGE. Insulin influenced phosphorylation of two membrane proteins of mol wt 95 kDal and 15 kDal. Phosphorylation of the 15 kDal protein was increased by insulin and could be attributed to stimulation of protein kinase activity. This effect was enhanced by micromolar concentration of GTP and to a lesser extent by Gpp(H)p. After ADP-ribosylation of the membrane in the presence of cholera toxin and NAD^+, this insulin effect was abolished. The 95 kDal protein was extremely rapidly phosphorylated reaching a maximum within 400 ms. In the absence of insulin, the protein was phosphorylated on serine residues. After insulin treatment, there was a switch to phosphorylation of tyrosine residues and this effect was enhanced by GTP. The polypeptide has been identified as the β-subunit of the insulin receptor. This work strongly indicates that insulin effects on membrane protein phosphorylations are mediated by GTP-regulatory proteins.

562. Conventional treatment of Type 1 (insulin-dependent) diabetes just prevents metabolic catastrophe

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To determine the efficacy of conventional insulin therapy metabolic control and presence of late complications (physical check-up/ophthalmoscopy/proteinuria/creatinine) were evaluated in Type 1 diabetic patients treated with insulin and diet either by a University Hospital outpatient service (UH: $n = 137$; aged 38.5 ± 16.1 years; duration of diabetes 12.8 ± 9.3 years) or in a rural setting (RS: $n = 73$; aged 38.3 ± 20.8 years; duration of diabetes 12.7 ± 9.5 years). Checking both the percentage of patients displaying an abnormal metabolic state (blood glucose > 8.9 mmol/l; HbA_{1c} > 5.8%; glycosuria > 3.5 g/24 h; creatinine > 1.3) and presence of late diabetic complications, poor therapeutic efficacy was observed (UH/RS: blood glucose 70.7/86.3%; glycosuria 79.3/72.6%; HbA_{1c} 81.4/95.5%; HDL-cholesterol 4%/36.9%; creatinine 12/19%; proteinuria 4.9/12.7%; retinopathy 36.8/40.8%; peripheral neuropathy 18/21%; macroangiopathy 12/16%). This situation was also reflected by the hospitalization period per patient, which increased from 33 days during the first to 98 days during the fifth 5-year period of Type 1 diabetes. These data demonstrate that both in a 'diabetes centre' and even more so in a rural area the majority of diabetic patients appears almost untreated if strict metabolic and clinical criteria are applied. We conclude that conventional insulin treatment prevents acute metabolic catastrophe only, but is unable to control the metabolic state or to avoid the development of late complications in more than one-third of Type 1 diabetic patients.

563. Stimulation of glucose uptake by muscle contraction does not require the presence of insulin

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From studies of perfused rat muscles, it has been concluded that contraction-induced increase in glucose uptake requires the presence of a permissive amount of insulin. We investigated this claim using the perfused rat hind limb preparation. Rats were made diabetic (125 mg streptozotocin/kg body weight), and studied either 72 h later (group 1) or maintained on insulin for 2 weeks and then studied 72 h after cessation of insulin therapy (group 2). Only diabetic rats with plasma insulin levels too low to measure were used. Glucose uptake was determined in the basal state, during 20 min of sciatic nerve stimulation and during 15 min post-stimulation. Glucose uptake by resting muscle was found to be lower in group 1 compared with control rats (3.5 ± 0.5 versus 6.5 ± 0.7 μmol·g^{-1}·h^{-1}, $p < 0.01$). Despite the absence of insulin in the perfusion medium, muscle contraction resulted in large increases in glucose uptake both in the diabetic and control rats. During stimulation the uptake was increased sevenfold (group 1) and persisted elevated sevenfold (group 1) and fivefold (group 2) during the post-stimulation period. In absolute terms, there were no significant differences in glucose uptake between the diabetic and control rats, either during stimulation or post-stimulation. It is concluded that stimulation of glucose uptake by contractile activity does not require the presence of insulin.

564. Continuous intraperitoneal insulin infusion in Type 1 (insulin-dependent) diabetes: comparison of kinetics with intravenous insulin infusion

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Continuous intraperitoneal insulin infusion (CIPII) is the favoured route for insulin delivery with implantable pumps. With a view to glucose-controlled devices we studied kinetics of plasma free insulin (FI) in six Type 1 diabetic patients during different IP and intravenous (IV) infusion profiles using an externally portable device (Promedos E1, Siemens). 600 kcal meals of identical composition (48 g carbohydrate) were given and the same insulin dose was infused either as a bolus (B) or a 1-h square wave (SW), or B + SW. Four healthy subjects served as controls. In contrast to IV infusion, during CIPII FI levels were within the normal range, peaks occurring at 20 min after B, 70 min after SW and 50 min after B + SW, but basal levels were not attained within 3 h. Post-prandial excursion of blood glucose was near-normal only under IP B + SW. Three hours after ceasing the IP basal-rate infusion there was still a significant level of FI resulting in a slow increase of blood glucose (2.5 ± 0.6 mmol/l). In addition, after moderate exercise a marked drop of blood glucose was seen during CIPII. Therefore, peripheral hyperinsulinism can be avoided only by IP infusion systems, but the delay in insulin absorption from the peritoneum makes rapid changes of infusion rates impossible. In conclusion, CIPII may be suitable for program-controlled devices, but response seems not to be rapid enough for use in glucose-controlled insulin infusion systems.

565. Patients' experience with, and acceptance of, multiple insulin injections using cartridge-packed insulin in a novel injection pen

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An insulin pen (Novopen) incorporating cartridge-packed U100 human Actrapid was used in a group of Type 1 (insulin-dependent) diabetic patients to assess its acceptance and suitability in a multiple-dose injection regimen. 31 patients entered; one withdrew after 3 weeks' pen therapy. Patient characteristics were (mean ± SD

(range)): 20 males: 11 females; age 31.4 ± 11.2 years (16.5–57.0); duration of diabetes 12.3 ± 10.0 years (0.8–38); total daily insulin dose (52 ± 18 U (18–80). Three patients were C-peptide-positive. Following one month's treatment with twice-daily human Actrapid/human Monotard, patients changed to three pre-prandial soluble insulin injections using a Novopen with an evening injection of human Monotard. Insulin dose, control and dietary intake were monitored and patient attitudes to the regimen assessed by a clinical psychologist. There was an elective dose reduction on changeover. However after 3 weeks multiple daily injections the total dose returned to baseline [55 ± 15 U (run-in) versus 53 ± 13 U (3 weeks Novopen)] and was then maintained. Control before and at 8 weeks on pen therapy was unchanged (mean HbA_{1c} 11.8 ± 3.1% versus 11.6 ± 2.4%). Patients stressed the convenience and increased flexibility the pen gave; 27 (90%) chose to continue multiple injections using Novopen. The Novopen provides a convenient and acceptable means of multiple injections.

566. Prostaglandyl-inositol cyclic phosphate, a second messenger for insulin

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The intracellular regulator cyclic AMP antagonist (cPIP), which is well suited to be a mediator of insulin action, inhibits cyclic AMP-dependent protein kinases and activates phosphoprotein phosphatase. Thus, by regulating the equilibrium between the phospho- and dephosphoforms of interconvertible enzymes, cPIP, for example, activates the mitochondrial pyruvate dehydrogenase fivefold and completely inhibits the phosphorylase. cPIP has been isolated after hormonal stimulation from rat hepatocytes or pig livers at a concentration of 0.6×10^{-7} mol/l. cPIP also occurs in other tissues like heart, fat, muscle, kidney and brain, thus suggesting that cPIP is a ubiquitous regulator. Purification is achieved by gel filtration, anion exchange-, affinity- and high pressure liquid chromatography. cPIP is different from reported putative second messenger peptides. The structural components of cPIP are prostaglandin E_1, inositol and phosphate. These have been identified by degradation of cPIP and mass spectrometric identification of the fragments. Furthermore, radioactive labelled components are incorporated into cPIP. From chemical derivatisation experiments a structure for cPIP can be inferred where the phosphate is linked to an inositol and this inositol-phosphate to the prostaglandin by its C-15 hydroxyl group.

567. Interactions between glucagon and other counter-regulatory hormones during exercise in diabetes

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Ten partially insulin-deficient, alloxan-diabetic dogs were exercised for 90 min (100 m/min, 12°) with or without somatostatin (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Before exercise, insulin was 63% lower (6.3 ± 2.4 mU/l), glucose metabolic clearance 59% lower (1.41 ± 2.8 $ml \cdot kg^{-1} \cdot min^{-1}$), glucagon 297% higher (695 ± 359 pg/ml), plasma glucose 269% higher (13.5 ± 2.9 mmol/l) than normal. Glucose production (Ra, 4.00 ± 1.30 $mg \cdot kg^{-1} \cdot min^{-1}$) and utilization (Rd, 4.55 ± 1.38 $mg \cdot kg^{-1} \cdot min^{-1}$), adrenaline (93 ± 41 pg/ml), noradrenaline (204 ± 95 pg/ml) and cortisol (4.3 ± 3.6 μg/dl) were normal. In the diabetic dogs, during exercise, increments in Ra and Rd (Δ5.4 ± 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$) were normal, and glucose fell only marginally (11%). Glucagon (Δ765 ± 117 pg/ml) and cortisol (Δ5.5 ± 2.8 μg/dl) rose 2.5-fold more than in normal dogs, but interestingly, insulin did not change during exercise. With glucagon suppression (Δ506 ± 153 pg/ml), the Ra increment was virtually abolished. Since somatosttatin did not diminish the glucose metabolic clearance increment, the drop in glucose (Δ5.3 ± 1.8 mmol/l) was much greater than in normal dogs. Despite the rapid decline in glycaemia, increments in adrenaline, noradrenaline and cortisol were not excessive. In conclusion, as in normal dogs, glucagon suppression during exercise resulted in a decline in glycaemia. However, glucose levels were never in the hypoglycaemic range, thus no counter-regulatory response to glucagon lack, and the full impact of glucagon suppression on Ra was revealed. Thus, in diabetes, as in health, glucagon is the primary regulator of Ra during exercise.

568. Characterisation of a mixture of biotinylated insulin

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To develop probes to characterise further the interaction of insulin with its receptor, we prepared a mixture of biotinylated-insulin derivatives. Purified porcine insulin was reacted with biotin-n-succinimide ester, and the resultant biotinylated-insulin derivates were separated from the reaction mixture by gel filtration. This mixture was 35% as potent as insulin in a radioreceptor assay and had a similar potency in the stimulation of glucose oxidation in rat adipocytes. After iodination of the mixture, anti-insulin antibodies and anti-biotin antibodies were equally efficient in immunoprecipitating radioactivity. Avidin-agarose depleted 90% of the insulin activity of the mixture. These data suggest a high biotin incorporation into insulin. When the iodinated biotinylated insulin mixture was cross-linked to the insulin receptor, the biotin-^{125}I-insulin complex could be immunoprecipitated with antibodies directed against biotin, insulin or insulin receptor. High performance liquid chromatography was used to fractionate the heterogenous derivatives. Two major peaks were purified. Both of these peptides retained receptor binding and biological activity and gave curves parallel to the porcine insulin standard in a radioimmunoassay. These insulin derivatives may provide tools in studying the interaction of insulin with its receptor.

569. Myocardial infarction size in diabetes

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Diabetic patients have a higher risk of mortality after a first myocardial infarction compared with non-diabetic subjects. Myocardial infarction size is recognized as the most important predictor of survival. This study was designed to test the hypothesis that myocardial infarctions are larger in diabetic than non-diabetic patients. Forty-four diabetic patients (all first myocardial infarctions) were matched by age, sex, site of infarct and year of hospitalization with 44 non-diabetic patients. Records of these admissions to hospital were obtained (1978–1981) and ECG readings were taken from day of discharge, or day 14 after admission. The size of infarcts was determined by the QRS scoring method. Overall, the QRS score was significantly higher among diabetic than non-diabetic subjects (6.66 versus 4.86, $p < 0.01$). This was true for inferior (6.79 versus 4.70, $p < 0.05$) but not for anterior infarcts (6.50 versus 5.05) although the same trend was present. Diabetic women had significantly higher scores than non-diabetic women (7.13 versus 4.53, $p < 0.05$) but there were no significant differences between diabetic and non-diabetic men (6.41 versus 5.03). These results suggest that the larger myocardial size of infarcts among diabetic patients could be a major determinant of their higher cardiovascular mortality.

570. Discrimination between glucose- and K^+-sensitive pools of calcium in pancreatic β cells with digitonin permeabilization

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β-cell-rich pancreatic islets from ob/ob mice were used for discrimination between the pools of calcium sensitive to glucose and excessive K^+ using a digitonin permeabilization technique. Permeabilization was either performed directly after loading of the islets with ^{45}Ca or after a subsequent 60-min chase period. Glucose- and K^+-depolarization preferentially stimulated ^{45}Ca uptake into a pool sensitive to a high concentration of digitonin and into one resistant to the detergent. The ^{45}Ca incorporated in response to glucose was more firmly bound within the cells since only 42% was released during the chase period compared with 57% K^+-incorporated ^{45}Ca. This difference between glucose and K^+ became very striking when the origin of the ^{45}Ca released was analyzed with the digitonin permeabilization procedure. After glucose-stimulated loading the pool sensitive to the highest concentration of digitonin only contributed to 1% of the ^{45}Ca released during the chase period. However, no less than 38% of the chase-sensitive ^{45}Ca originated from this pool after K^+-stimulated loading. When taking into account the release of organelle markers during digitonin permeabilization, the results suggest that the glucose-stimulated firm incorporation of ^{45}Ca into the pancreatic β cell is due to uptake into mitochondria.

571. Glycosylation is essential for the biosynthetic processing of the insulin receptor of IM-9 lymphocytes

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The insulin receptor is a glycoprotein. We have used tunicamycin, an inhibitor of N-linked glycosylation, to study the role of its carbohy-

drate moiety in IM-9 lymphocytes. Using cell labelling and immunoprecipitation techniques, we demonstrate that the receptor carbohydrate is N-linked and glycosylation is essential for its biosynthetic processing. After 24 h incubation with tunicamycin (0.5 μg/ml) insulin binding was reduced by 50–70%. This was paralleled by a proportional decrease in the concentration of the insulin receptor subunits (M_r 210K, 135K, 95K) determined by cell surface iodination, suggesting impaired receptor biosynthesis. Pulse chase studies with ^{35}S-methionine were performed to examine this possibility. In control cells, the 190K receptor precursor appeared at the end of the pulse and was processed to the mature receptor subunits (M_r 210K, 135K, 95K) with a $t_{½}$ of 90–120 min. After tunicamycin treatment a 180K protein appeared at a similar time but disappeared without further processing with a $t_{½}$ of 30–60 min. Tunicamycin abolished labelling of the receptor with ^{3}H-glucosamine, a precursor of N- and O-linked glycoproteins. These data suggest that the insulin receptor contains only N-linked carbohydrate and that the M_r of the unglycosylated receptor is 180K. Furthermore, addition of carbohydrate appears to be essential for the processing of the receptor.

572. Transport and phosphorylation of glucose at the level of the plasma membrane in rat adipocytes

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Isolated adipocytes of the rat possess an insulin-sensitive glucose transport system. It is generally accepted that uptake of glucose occurs through facilitated diffusion. However, when glucose is incubated with pure membrane vesicles (characterised using electron microscopy and biochemical methods) and ATP, we found that glucose was phosphorylated at the level of the plasma membrane. Transport and phosphorylation are inhibited by specific transport inhibitors such as cytochalasin B and phlorizin. Phosphorylation of glucose is stimulated by Mg^{2+} (maximal at 6 mmol/l) and is inhibited by Ca^{2+} (up to 30%). The phosphorylation is pH-dependent, with an optimum at pH 8.2. The glucose analogue 2-deoxyglucose competitively inhibits transport and phosphorylation in contrast to 3-0-methylglucose, which has no effect. Phosphorylation is markedly inhibited by glucose-6-phosphate, but not by 2-deoxyglucose-6-phosphate, suggesting the involvement of a hexokinase. Treatment of the membranes with Triton X-100 and glucose-6-phosphate decrease the phosphorylation, implying that phosphorylation is catalysed by a hexokinase associated with the membrane. The present data demonstrate that, besides facilitated diffusion, a transport-associated-phosphorylation mechanism may play a rôle in glucose uptake.

573. Autoantibodies to human insulin

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An immunospecific enzyme-linked immunosorbent assay (micro-ELISA) incorporating purified insulins was used to screen sera from insulin-naive, non-diabetic subjects with various autoimmune disorders, and identified 15 which bound insulin. Five bound human (H), porcine (P) and bovine (B) insulins but the remaining 10 bound exclusively H insulin, to a degree equivalent to 'moderate' binding in diabetic sera. Binding was isolated to the immunoglobulin fraction of the test sera, and was not observed in sera from 'non-autoimmune' subjects. 'Cold' H, P and B insulins each totally displaced, in a dose-dependent manner, the five sera which bound all three insulins, but only H displaced the other 10, P and B having no effect in concentrations up to 1000 U/l. The insulin-binding immunoglobulins in all 15 sera were essentially IgG and many possessed a single (K or λ) light chain. Insulin antibodies in sera from insulin-treated diabetic patients, on the other hand, were always ditypic (both K and λ light chains) and bound all three insulins equally. Autoantibodies to insulin are therefore often monotypic, occur only in the context of established autoimmunity and in many cases relate exclusively to the single amino-acid substitution (B 30) which distinguishes H from P insulin.

574. Insulin gene polymorphism: a racial study

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Restriction enzyme analysis has identified a DNA polymorphism adjacent to the human insulin gene, due to variable insertions of a repeated 14 base pair nucleotide sequence. These insertions are not random and exist in three predominant classes: class 1 (approximately 40 repeats), class 2 (approximately 95 repeats) and class 3 (approximately 170 repeats). Disease associations (including diabetes and atherosclerosis) have been reported with class 1 and class 3 inserts, although early studies were conflicting. This is possibly due to the inclusion of individuals of differing racial origin in prevalence analysis. We have therefore studied this DNA polymorphism in normolipaemic, normoglycaemic subjects within differing ethnic groups using Southern blotting techniques. The allele frequencies for class 1, 2 and 3 inserts respectively were: Caucasoid ($n=52$) 0.67, 0.00, 0.33; Asian Indians ($n=23$) 0.82, 0.00, 0.18; Japanese ($n=24$) 0.94, 0.00, 0.06; Chinese ($n=21$) 0.86, 0.00, 0.14; Africans ($n=31$) 0.55, 0.24, 0.21; and West Indians ($n=16$) 0.53, 0.25, 0.22. These varying allele frequencies in groups of differing racial origin emphasise the importance of racial homogeneity when assessing disease associations in different populations.

575. Smoothing of blood glucose profile by a new α-glucosidase-inhibitor (BAY m 1099): a short-term study

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The therapeutic problem in most Type 2 (non-insulin-dependent) diabetic patients is the post-prandial blood glucose (BG) increase. Thus, slowing the resorption of carbohydrates from the intestine by α-glucosidase-inhibitors seems to be a useful therapeutical tool. We studied the effect of the new α-glucosidase-inhibitor, BAY m 1099, a resorbable 1-desoxynojirimycine, on 24-h-BG profiles of 12 sulphonylurea treated Type 2 diabetic patients using a placebo-controlled double-blind cross-over design. The study consisted of three phases (A, B, C) of 4 days each: the patients took twice daily 50 mg BAY m 1099 either in phase A or C and placebo in both other phases, phase B served as wash-out-phase. At the beginning and at the end of each phase BG profiles (21 values/24 h) were taken. Fasting and mean BG levels did not change significantly. The standard deviation of daily mean BG, however, was lower with BAY m 1099 (1.57 ± 0.52 versus 2.11 ± 0.47 mmol/l; $p<0.02$). BAY m 1099 significantly reduced the post-prandial rise in BG 45, 60 and 75 min after the first breakfast and 45 and 120 min after dinner ($p<0.05$ or less), the daily BG maximum was reduced from 12.06 to 9.74 mmol/l. Oral administration of BAY m 1099 resulted in a significant smoothing of 24-h BG profiles and seems useful as an adjunct to sulphonylurea treatment in Type 2 diabetes.

576. Exaggerated effect of low-dose angiotensin II on the high glomerular filtration rate of diabetes

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The effect of intravenous angiotensin II (AII) on glomerular filtration rate (GFR) was studied in insulin-dependent diabetic patients, six with elevated and four with normal GFR. GFR was measured using constant insulin infusion during infusion of AII at 1.5 $ng \cdot kg^{-1} \cdot min^{-1}$ or saline control. GFR in the hyperfiltering group fell from 159 ± 11 to 132 ± 22 (−17%; $p<0.02$) and returned to 150 ± 18 ml/min per 1.73 m^2 after stopping AII. In the normofiltering group, GFR fell from 125 ± 5 to 114 ± 5 ml/min per 1.73 m^2 (−8%; $p<0.05$) during AII with some overshoot after stopping it (138 ± 7 ml/min per 1.73 m^2). The GFR fall during AII was significantly greater in the hyperfiltering than the normofiltering group (26 ± 17 versus 11 ± 6 ml/min per 1.73 m^2; $p=0.05$). There was a significant negative correlation between the 'rebound' rise in GFR after AII and baseline GFR ($r=-0.63$, $p<0.05$). Mean arterial blood pressure was similar at baseline in both groups, and rose slightly but not significantly (3–4 mmHg) during AII. Insulin-dependent diabetic patients with high GFR show exaggerated glomerular responsiveness to suppressor doses of AII. Abnormalities of the intrarenal renin-angiotensin system or its interactions may contribute to the high GFR of diabetes.

577. Effect of synthetic rat C-peptide II, pork C-peptide and biosynthetic human C-peptide on the rate of disappearance of ^{125}I-insulin in rat plasma

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Synthetic rat C-peptide administered to rats diminished the glucose-induced increase of insulin secretion, but did not significantly alter glucose tolerance. In diabetic rats, C-peptide significantly increased and prolonged the hypoglycaemic effect of exogenous insulin. This work has been performed to estimate the effect of synthetic rat C-peptide II, pork and biosynthetic human C-peptide on the rate of disappearance of ^{125}I-insulin in rat plasma. C-peptide was infused to portal

vein of anaesthetized male Wistar rats (180–220 g) throughout the experiments. Biologically active insulin (40 μg/kg) was given intraportally at 10 min as an intravenous bolus. Samples of blood were taken from the femoral artery for glucose and trichloroaetic acid precipitable radioactivity determination. Effects of rat C-peptide II (2.5 or $10\,\mu g \cdot kg^{-1} \cdot 30\,min^{-1}$), pork and biosynthetic human ($10\,\mu g \cdot kg^{-1} \cdot 10\,min^{-1}$) were tested. The infusion of rat C-peptide II caused a dose-dependent increase rate of disappearance of ^{125}I-insulin from plasma. Half-life of ^{125}I-insulin in plasma was 3.1 min and in the presence of rat C-peptide II was 2.5 min in the lower ($p < 0.05$) and 2.0 min in the higher concentration ($p < 0.01$). Pork and human C-peptide were without effect. None of the C-peptides had any effect on blood glucose. The increased rate of disappearance of ^{125}I-insulin in plasma indicates a possible connection between this effect and insulin degradation.

578. Lack of correlation between nutrient-induced changes of cytosolic pH and insulin release

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Changes in cytosolic pH (pH_i) have not yet been accurately monitored in insulin-releasing cells, because the probes used hitherto do not remain strictly trapped in the cytosol. In contrast, the new fluorescent pH-indicator 2′, 7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF) remains in the cytosol for several hours following intracellular hydrolysis of its lipophilic acetoxymethylester. BCECF fluorescence was monitored either in RINm5F cell suspensions using a fluorometer or in adult rat islet monolayers using a microscope attached to a cytofluorometer. Membrane potential (monitored with bisoxonol) and insulin release were measured in parallel. Resting pH_i in RINm5F cells was 6.95 ± 0.01 ($n = 13$). D-glyceraldehyde (10 mmol/l), which depolarizes the cells and stimulates insulin release, caused a rapid and reversible decrease of pH_i (maximal decrease 0.11 ± 0.01, $n = 5$). Glucose (16.7 mmol/l) elicited a comparable decrease of pH_i in the islet monolayers. However, it is unlikely that intracellular acidification mediates the action of glyceraldehyde, since L-lactate (20 mmol/l), while inducing similar acidification, failed to reproduce the effects on membrane potential and insulin release. Moreover, pH_i was not changed by alanine but increased by leucine-methylester, while both agents (10 mmol/l) depolarize and stimulate insulin release. In conclusion, glyceraldehyde and glucose decrease pH_i, but the acidification does not couple nutrient recognition to the insulin release process.

579. C-peptide responses to insulin infusion in obese subjects

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In normal subjects inhibition of insulin secretion occurs in response to a lowering of blood glucose concentration. In obese subjects it is unclear whether similar suppression occurs independent of circulating insulin concentration. Six normal men (age range 22–39 years; mean 31 years) and six normal women (age 25–35 years; 31 years) all <110% ideal body weight and six obese subjects (age 36–47 years; 41 years) >200% ideal body weight were infused in consecutive 1 h periods with saline, actrapid insulin $0.005\,U \cdot kg^{-1} \cdot h^{-1}$, actrapid $0.01\,U \cdot kg^{-1} \cdot h^{-1}$, and actrapid $0.05\,U \cdot kg^{-1} \cdot h^{-1}$. Fasting blood glucose was similar in the three groups. Fasting plasma insulin and C-peptide concentrations were significantly higher in obese subjects (13.0 ± 3.0 mU/l and 5.1 ± 1.2 μg/l, respectively; $p < 0.001$). Circulating insulin concentrations during insulin infusion were higher in obese subjects but the fall in blood glucose concentration was less (to 3.3 ± 0.4 mmol/l) than in both normal men and women (to 2.3 ± 0.2 mmol/l). C-peptide concentrations were greater throughout the insulin infusion in obese subjects but the decrease was similar in all three groups. These results suggest that in obese subjects suppression of insulin secretion may be due in part to circulating insulin concentrations.

580. Production in vitro of islet cell antibodies in insulin-dependent diabetes

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Production in vitro of islet cell antibodies (ICA) by peripheral blood mononuclear leucocytes (MNL) from patients with Type 1 (insulin-dependent) diabetes was investigated using Pokeweed mitogen (PWM) as a non-specific stimulator. Blood samples were collected from 12 Type 1 diabetic patients, 10 Type 2 (non-insulin-dependent) diabetic patients and 10 normal subjects. MNL were isolated from heparinized blood by Ficoll Conray gradient centrifugation. Cells (1×10^6) were cultured in 1.5 ml of culture medium (RPMI 1640) containing 10% fetal calf serum and 1% PWM, and the culture supernatants were harvested on day 10 for determination of ICA. The supernatants were precipitated by 50% saturated ammonium sulphate and the dissolved precipitates were lyophilized after extensive dialysis. These gammaglobulin fractions of the supernatants were diluted to appropriate concentrations before ICA determination. ICA was measured by a method using peroxidase-labelled Protein A. ICA was detected in culture supernatants in five out of 12 Type 1 diabetic patients and production of ICA in vitro correlated significantly with serum ICA. None of MNL obtained from the 10 Type 2 diabetic and the 10 normal subjects produced ICA in vitro. The production of ICA in vitro will provide invaluable probes in the investigation of Type 1 diabetes.

581. Effects of exogenous insulin on the surface topography of isolated pancreatic islet cells from diabetic mice

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Islets of diabetic and normal C57BL mice, isolated by collagenase digest, were incubated for 1 h at 37 °C in glucose (3 mmol/l). Diabetic islets were also incubated in glucose (3 mmol/l) containing either 50 mU/l or 150 mU/l mouse insulin. Both normal and diabetic islets were then perifused for 20 min with glucose (3 mmol/l or 15 mmol/l). The islets were immediately prepared for scanning electron microscopy. β cells within the islet mass were identified as round or oval cells with a mean diameter of 10 μm. Normal and diabetic cells perifused with glucose (3 mmol/l) appeared relatively smooth with few cytoplasmic projections (blebs) or stomata (pits). Stimulated normal cells showed a marked increase in blebs/unit area, concomitant with a 6-7-fold increase in insulin release. Untreated diabetic cells exhibited no response to glucose (15 mmol/l) in either surface blebbing or insulin release. Diabetic cells treated with insulin (50 mU/l ~150 mU/l) showed a response to glucose (15 mmol/l), both in increased cell surface blebbing and insulin release when compared with untreated diabetic cells from the same digest. It is suggested that cell surface blebbing is related to emiocytosis, and that pre-incubation with insulin increased blebbing in diabetic β cells.

582. Insulin-mediated glucose metabolism is unrelated to free insulin levels in Type 1 (insulin-dependent) diabetes: evidence for biological activity of insulin antibodies

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To evaluate the effect of insulin antibodies and plasma free insulin levels on glucose metabolism in vivo, we performed euglycaemic clamps (1 mU) in 55 Type 1 diabetic patients and 18 healthy subjects. During the clamp, steady-state free insulin (SSFI) levels averaged 71 mU/l (range 35–120 mU/l) in the diabetic patients and 82 mU/l (range 70–120 mU/l) in the control subjects ($p < 0.01$). SSFI was inversely related to insulin antibody level in diabetics ($r = -0.70$, $p < 0.001$). The diabetics were grouped based on quartiles of SSFI: groups A < 59 mU/l, B 59–69 mU/l, C 70–80 mU/l, D > 80 mU/l. These groups were comparable with respect to age, sex, weight, HbA_1, C-peptide and insulin dose. Despite varying SSFI levels (47 ± 1, 65 ± 1, 74 ± 1, 96 ± 3 mU/l for groups A, B, C, D, respectively), the rates of glucose metabolism (M-values) were comparable (5.33 ± 0.53, 4.82 ± 0.46, 4.67 ± 0.30, $4.65 \pm 0.46\,mg \cdot kg^{-1} \cdot min^{-1}$ for groups A, B, C, D, respectively) and 36–44% reduced compared with control subjects ($8.27 \pm 0.43\,mg \cdot kg^{-1} \cdot min^{-1}$, $p < 0.001$). To exclude inherent differences in insulin sensitivity between the groups, insulin binding and action (D-^{14}C-glucose transport, oxidation and lipogenesis) were measured in adipocytes of two subgroups with different antibody and SSFI ($p < 0.01$) levels but similar M-values. No difference was found in insulin binding or action in vitro. In conclusion: (1) low free insulin levels during the clamp are due to antibodies; (2) insulin resistance in Type 1 diabetes is unrelated to insulin antibodies; (3) comparable glucose metabolism regardless of free insulin levels suggests a direct insulin-like effect of insulin antibodies.

583. Evidence for an association independent of glycaemic control between developing neuropathy and microangiopathy in young diabetic patients

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In cross-sectional studies subclinical abnormalities of nerve function in young diabetic patients are more severe in those with poor glycaemic control. Recent evidence has favoured a predominantly metabolic aetiology for diabetic neuropathy. We have now investigated whether poor glycaemic control is the principal influence on further evolution of subclinical neuropathy. Seventy-five teenage (16–19 years) diabetic patients have been studied prospectively for 30 months. Mean values for motor and sensory peripheral nerve electrophysiology (PNEP) deteriorated significantly in the leg, but not the arm; mean values for five cardiovascular autonomic function tests (CAFT) were unaltered. Considered separately, high HbA_1 initially and at review, duration of diabetes, retinopathy at review and CAFT at review were each significantly ($p<0.01$) related to deteriorating PNEP (leg). However, although poor glycaemic control correlates with impaired nerve function and is the principal determinant of the development of retinopathy in the diabetic patients, when all the potentially explanatory variables above are considered simultaneously in a multiple regression analysis, the strongly dominant relationship with deteriorating PNEP (leg) is retinopathy ($p<0.00001$) rather than HbA_1. This surprisingly close-link between early evolutionary stages of these two diabetic complications suggests a pathogenic interrelationship independent of any direct effect of poor glycaemic control on either.

584. Proinsulin hypersecretion in a case of insulin autoimmune syndrome

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Circulating insulin antibodies and pancreatic β-cell function were determined bimonthly over one year in an elderly female patient, without history of known immunization, who suffered initially from mild hypoglycaemic episodes. The insulin-binding capacity of IgG declined progressively from 1.29 to 0.03 U/l. The binding of ^{125}I-mono-A14-bovine, porcine and human insulin to the patient's antibodies was equally strong. Repeated oral glucose tolerance tests showed borderline glucose levels despite excessive secretory responses of immunoreactive free insulin, C-peptide and proinsulin. Multiple fasting serum samples, both from the patient and from Type 2 diabetic patients with insulin-binding IgG (range 0.14–8.04 U/l) were extracted with acid ethanol for fractionation by gel filtration. When expressed as a percentage of the total insulin concentration, the proinsulin-like material in the patient with autoantibodies averaged 44.2% (range 35–52%) compared with 19.1% (range 8–26%) in the diabetic group. The significant elevation of the proinsulin peak in the patient persisted when insulin antibodies became undetectable and was characterized by a high-molecular-weight C-peptide immunoreactivity. These observations suggest that the production of proinsulin-like components may be involved in the development of the insulin autoimmune syndrome.

585. Noradrenaline decreases insulin binding in human fat cells in vitro

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Catecholamines may induce insulin resistance, but the mechanism(s) involved in this phenomenon are not yet completely understood. In an attempt to improve our knowledge on this topic we investigated whether noradrenaline (NA) may affect insulin binding in adipose tissue in vitro. Subcutaneous abdominal adipose tissue was obtained from patients undergoing surgery ($n=8$); tissue samples were cut in small fragments (about 25 mg each) and pre-incubated at 37 °C in medium 199 (5.6 mmol/l glucose, 4% bovine serum albumin) both without hormone addition and NA at maximal lipolytic concentration (5×10^{-5} mol/l). After 24 h of pre-incubation, adipose cells were isolated by collagenase digestion, insulin binding tested at equilibrium (22 °C; 45 min incubation) for each pre-incubation condition and competition curves generated. Insulin binding was significantly lower in the NA pre-incubated cells at low insulin concentration in comparison with controls. These preliminary data suggest that NA can influence insulin binding in a target tissue for insulin. To explain such a finding we may assume as a working hypothesis that a NA-induced increase in a non-esterified fatty acid concentration in the pre-incubation medium could be involved.

586. Regulation of glucose transport in heart of lean and genetically obese *fa/fa* rat

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Glucose transport is stimulated by a translocation of specific glucose transporters from an intracellular pool to the plasma membrane. The aim of this study was to measure glucose transporters in plasma and microsomal membranes of heart of lean and obese rats, using the D-glucose inhibitable cytochalasin B binding assay. In lean animals and under basal conditions, 90% of transporters were located in the microsomes. When insulin and higher perfusion pressure or glucose were applied, a decreased number of transporters in microsomes together with an increase of those in plasma membranes were observed. Total number of transporters (plasma and microsomal membranes) remained constant under all conditions tested. In obese rats under basal conditions, a diminished number of transporters was observed in both plasma and microsomal membranes. Decreased total transporters of obese hearts was the likely cause of the lesser number of transporters measured in their plasma membranes following application of the stimuli mentioned. In conclusion, (a) in normal heart, translocation of glucose transporters from an intracellular pool to the plasma membrane is triggered by seemingly unrelated effectors such as insulin, perfusion pressure and glucose per se; (b) this system is altered in heart of obese rats, in keeping with their decreased ability to transport glucose.

587. Spontaneous endogenous hypertriglyceridaemia independent of obesity in a rat model

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Male Sprague Dawley IVA-SIV rats were studied and compared with male Sprague Dawley Charles River rats of the same age (7 weeks). They showed similar body weight (216 ± 3 versus 211 ± 2 g) and daily food intake. Nevertheless fasting hypertriglyceridaemia (2.07 ± 0.1 versus 1.49 ± 0.1 mmol/l, p 0.001) was present, which was associated with high fasting plasma glucose (6.4 ± 0.2 versus 4.6 ± 0.1 mmol/l, p 0.001), plasma insulin (34.5 ± 4.8 versus 18.7 ± 2.15 mU/l, p 0.01) and plasma non-esterified fatty acid levels (1.08 ± 0.07 versus 0.56 ± 0.05 mmol/l, p 0.001). An oral glucose load (180 mg/100 g body weight) produced evidence of reduced glucose tolerance and significantly higher insulin secretion. The VLDL-triglyceride turnover indicated a higher triglyceride production rate (2.4 ± 0.2 versus 1.8 ± 0.1 mg/min per rat, p 0.01), significantly correlated with plasma triglyceride ($r=0.58$ p 0.01). Lipoprotein lipase activity in both adipose tissue and muscle was not significantly different in the two groups of rats. All these metabolic variables were significantly altered at 3 weeks of age. IVA-SIV rats therefore gave a model of spontaneous endogenous hypertriglyceridaemia independent of obesity. The hypertriglyceridaemia, induced by increased hepatic VLDL-triglyceride secretion, is associated with hyperinsulinaemia, insulin resistance and high non-esterified fatty acid levels.

588. Species differences in the insulin-binding-action linkage in human and rat adipocytes: studies with sulphated insulin

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Rat adipocytes have been intensively investigated with respect to insulin binding and action, and such results are often extrapolated to conclusions about human adipose tissue. During a comparison of sulphated insulin and unmodified insulin, major differences in the insulin receptor-action linkage in rat and human adipocytes were observed. There was no difference between sulphated and unmodified insulin in competition for binding sites on either human or rat adipocytes in terms of biologically defined units (half-maximal displacement at 9.6 ± 2.9 versus 9.2 ± 2.3 mU/l for human and 178 ± 26 versus 190 ± 43 mU/l for rat adipocytes). The biological effects of sulphated insulin on human adipocytes were indistinguishable from those of unmodified insulin. However, in rat adipocytes sulphated insulin was markedly less potent. Half-maximal stimulation for initial glucose transport rates occurred at 28.3 ± 11.3 mU/l (sulphated insulin) versus 5.5 ± 2.0 mU/l (unmodified insulin, $p<0.05$) for lipogenesis at

108 ± 35 versus 7.42 ± 2.38 mU/l ($p < 0.025$), for glucose oxidation at 134 ± 33 versus 5.56 ± 1.11 mU/l ($p < 0.01$) and half-maximal inhibition of lipolysis at 1.21 ± 0.39 versus 0.26 ± 0.01 mU/l ($p < 0.025$). Hence differing potencies of sulphated insulin were observed in human and rat adipocytes despite equivalent receptor binding. This implies differences in the post-binding steps of insulin action in adipocytes of the two species.

589. Insulin administration in gestational diabetes may influence fetal β-cell function, macrosomia and hypoglycaemia in neonates

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Since the best treatment in gestational diabetes (GD) is not yet established and GD may influence perinatal outcome, we investigated the following groups of women: 55 with GD with fasting blood glucose < 5.8 mmol/l treated by diet (group A), 41 with fasting blood glucose > 5.8 mmol/l on diet (group B) and 57 with fasting blood glucose > 5.8 mmol/l on diet plus insulin (group C). GD was diagnosed between weeks 24 and 34. Amniotic fluid insulin and C-peptide were significantly higher in group B than groups A and C [23.6 ± 8.6 versus 6.0 ± 0.6 and 6.4 ± 2.1 mU/l ($p < 0.001$) and 2.9 ± 0.4 versus 1.7 ± 0.7 and 1.2 ± 0.2 pg/ml ($p < 0.001$; mean ± SEM)]. Birth weights of fetuses from group B were significantly higher than group A (4095 ± 138 versus 3204 ± 76 g; $p < 0.001$). In contrast, birth-weights of group C were less (3459 ± 85 g; $p < 0.05$) and did not differ from group A. Children delivered to mothers in group B exhibited a high percentage of hypoglycaemia (61%) compared with 27% in group C and 11% in group A. Our results demonstrate that insulin administration once or twice daily in GD is necessary to prevent fetal β-cell function, macrosomia and hypoglycaemia in neonates. Moreover, fetal weight may be influenced by small fluctuations of blood glucose.

590. Differences in the pancreotropic behaviour of Coxsackie B4-virus isolates of patients

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Coxsackie B4-viruses (CB4) may play a role in the onset of diabetes mellitus. Therefore, the mechanism of action of this virus to pancreatic cells is of interest. C57Bl- and BALB/c-mice were infected by CB4-isolates characterized by SDS electrophoresis from patients with and without pre-treatment with streptocotocin (STZ, low dose). After 5 days in pancreas slices, CB4 as well as insulin were detected by a double immuno-fluorescence technique. From infected mice, pancreatic islets were isolated for measurement of insulin biosynthesis and secretion. Islet and acinar cells from normal as well as with STZ-pretreated mice were prepared: measurement of viability (^{51}Cr-release), incubation with ^{3}H-labelled CB4. The 20 CB4 isolates show different protein compositions. Six isolates developed pancreatitis without showing insulitis. Only after pre-treatment with STZ and subsequent infection, two isolates were able to develop hyperglycaemia. Attachment of ^{3}H-CB4 to isolated islet cells of BALB/c mice was low in contrast to cells of C57Bl/KsJ mice. Exocrine cells in both cases bound ^{3}H-CB4. The biochemical structure and the tropism against the pancreas of CB4 isolates were different. These properties may be an explanation for the fact that each CB4 is not able to initiate diabetes.