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REPORTS FROM MEETINGS

The Third Canadian Workshop on Diabetes

Lipid Metabolism

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The Third Canadian Workshop on Diabetes, with 32 invited participants, took place on October 27 and 28, 1969, at the Mont Gabriel Lodge, Mont Gabriel, Quebec. The meeting was sponsored by Hoechst Pharmaceuticals, a Division of Canadian Hoechst Ltd. The message of welcome was given by the Vice-President and General Manager of Hoechst Pharmaceuticals, Dr. H.G. Giese. The opening remarks were delivered by the Honorary President, Dr. A.L. Chute, Dean of the Faculty of Medicine, University of Toronto. Dean Chute chaired the morning session of October 27.

The first paper was presented by the guest speaker Dr. P.P. Foà, Division of Research, Sinai Hospital of Detroit, on *Lipogenesis in the Chick Embryo Heart.* A unitarian hypothesis of insulin action proposes that the glycogenic, protein anabolic and lipogenic effects of the hormone depend upon its well known property of facilitating glucose entry into the cell. Indeed, in the epididymal fat pad and in the lactating mammary gland of the rat, insulin stimulates lipogenesis from acetate or pyruvate, only when glucose is present in the incubation medium (Winegrad and Renold). On the other hand, in the chick embryo heart, the lipogenic effect of insulin can be demonstrated also in the absence of exogenous glucose (Foà *et al*). Similarly, in the chick embryo heart, insulin stimulates protein synthesis and lipogenesis after 5 days of embryologic development or several days before the stage when an insulin-sensitive transport system for glucose can be demonstrated (Guidotti *et al.*). Furthermore, exogenous glucose does not appear to be essential for the action of insulin on the incorporation of labelled amino acids into protein of the rat diaphragm. Under the conditions of the present experiments, insulin stimulated the synthesis of total lipids and of phospholipids, but not that of cholesterol, cholesterol esters, mono- and di-glycerides. The stimulatory effect of insulin on lipid synthesis was blocked by 2-deoxy-glucose even when exogenous glucose or other substrates were supplied. On the other hand, 3-methylglucose did not abolish the effect of insulin even when no glucose was added to the incubation medium. These results support the hypothesis that the lipogenic effect of insulin is independent of its action on the transport of glucose through the cell membrane.

Dr. C.H. Hollenberg, Department of Medicine, McGill University, Montreal, presented a paper on *Regulation of Adipose Mass - Nutritional and Hormonal Factors.* Adipose tissue mass is controlled by two processes: a) formation of new fat cells, b) turnover of lipid in existing cells. Although there is considerable information concerning the latter process, less is known about factors regulating DNA synthesis in adipose tissue. To explore this problem tritiated thymidine was injected into rats, adipose tissue was removed at varying times after injection and the tissue separated into 2 components, mature fat cells and stromal vascular elements, by collagenase digestion. The specific activity of DNA in the two cell populations was then followed. Up to two days after thymidine injection almost all radioactive DNA was in the stromal fraction, whereas after this time the radioactivity in the DNA of mature fat cells increased markedly. These findings suggest that within the stromal fraction were primordial fat cells that required at least two days after completion of DNA synthesis to accumulate sufficient lipid to be harvested in the fat cell pool. Fasting practically abolished incorporation of DNA into cells destined to become adipocytes and had a similar effect on other stromal elements. Growth hormone and insulin enhanced thymidine incorporation into stromal cells other than pre-adipocytes. Although insulin expanded adipose mass, this expansion was due entirely to an increase in lipid content per fat cell, rather than an increase in fat cell number. When animals were refed after an interval of fasting, no acceleration of DNA synthesis in primordial fat cells was apparent. Repletion of adipose mass in this circumstance was accomplished by refilling of existing, but emptied, cells. These data indicate that although in the growing rat new fat cells are continually formed, abrupt changes in adipose mass result from an alteration in lipid content per fat cell, rather than from a change in the rate of formation of these elements.

Dr. W.J. Poznanski, Ottawa Civic Hospital, spoke on the *Effect of Insulin on Amino Acids and Carbohydrate Metabolism in Human Fat Tissue.* Adipose tissue of various species is known to participate actively in carbohydrate, lipid and protein metabolism, and to be sensitive to certain effects of insulin. More recently this was shown to be true also for human adipose tissue studied *in vitro.* Glucose and amino acid uptake in human adipose tissue was studied, and the effects of physiological levels of insulin upon it were determined. It was hoped that the question concerning alleged resistance of diabetic tissues to the action of exogenous and/or endogenous insulin might be resolved by studies *in vitro* on adipose tissue from diabetic as well as from non-diabetic persons.

Results indicated that subcutaneous adipose tissue obtained from patients undergoing abdominal surgery metabolized glucose and amino acids well in the test system. Insulin at physiological levels stimulated glucose uptake and metabolism as shown by increased incorporation of radioactivity from glucose-1-^{14}C into the tissue and by increased $^{14}CO_2$ and ^{14}C-lipid formation. Insulin had no effect or only a slight effect on amino acid uptake or metabolism when glycine-^{14}C, 2-amino-isobutyric acid-^{3}H or leucine-^{14}C was added. In fact, insulin depressed uptake of $^{14}CO_2$ production from leucine-^{14}C in most cases, particularly with higher concentrations of insulin (1000 μU/ml).

There was considerable variability in activity of tissues from different donors, both without added insulin and with 50 μU or 200 μU of insulin per ml of incubation mixture. However, all tissue specimens except two responded with increased glucose metabolism when insulin was added, and the increase with 200 μU was greater than with 50 μU. In comparing diabetic with non-diabetic tissues there was no evidence that diabetic tissues had a diminished ability to metabolize glucose. In general, adipose tissue from diabetic patients showed a greater response to added insulin than did normal tissues, although there was considerable overlap between groups. Other studies from this laboratory with tissues from diabetic and non-diabetic patients have suggested the possibility of a dissociation of the effects of insulin on glucose uptake as opposed to amino acid transport.

Dr. J.I. Kessler, Royal Victoria Hospital, Montreal, spoke on *Plasma Lipids and Lipoproteins in Diabetes* with special reference to the *Effect of Treatment on Plasma*

Lipoproteins and Lipoprotein Lipase Activity. The interrelationship between the metabolism of carbohydrates and lipids has been well substantiated. Until recently, however, because of the lack of clinically applicable methods for lipid determinations, most of the metabolic studies in diabetes were restricted to alterations in the utilization of carbohydrates. Evidence is accumulating that in diabetes the alterations in the metabolism of lipids may be as important as those of the metabolism of carbohydrates, and that frequently the abnormality in carbohydrate utilization may be preceded by pronounced alterations in the metabolism of lipids.

In the present study it was found that acute insulin deficiency, produced by pancreatectomy or alloxan, resulted in a marked impairment of the removal of triglycerides from the circulation. This was associated with a marked inhibition of the activity of adipose tissue lipoprotein lipase and inability to liberate lipoprotein lipase in the circulation. Insulin reverted these parameters to normal.

Plasma lipids and lipoproteins were determined in 246 diabetic patients (44 newly discovered, 23 with latent diabetes and 153 with previously diagnosed diabetes). The incidence of lipoprotein abnormality was 30.2%, which is significantly higher than the incidence of lipoprotein abnormalities in a control group of 182 subjects (16.5%). The most frequent lipoprotein abnormality was that of Type IV (16.6%). Type II hyperlipoproteinaemia was found in 7.3%, followed by Type III in 3.4%, Type I in 1.6% and Type V in 1.2%. Treatment with diet, oral hypoglycaemic agents or insulin reduced markedly the incidence of hyperlipoproteinaemia (23.6%). The effect of treatment was most pronounced on Type I, IV and V, with virtually no effect on Type II hyperlipoproteinaemia. A significant correlation was found between the incidence and the severity of hyperlipoproteinaemia on one hand and the degree of control and vascular complications on the other hand. These results tend to indicate that satisfactory control of diabetes can revert the lipoprotein abnormalities to normal in a significant percentage of the cases. The close relationship between the vascular complications of diabetes and hyperlipoproteinaemia may indicate that early control of diabetes may prevent these complications.

The afternoon session was chaired by Dr. J. Davignon, Institut de Recherches Cliniques de Montréal.

Dr. G. Steiner, Department of Medicine, University of Toronto, presented a paper entitled *Sympathetic Regulation of Adipose Tissue Metabolism.* The rôle of the sympathetic nervous system (SNS) in regulating adipose tissue metabolism was investigated. A convenient way of producing long-term sympathetic hyperactivity in rats is to acclimate them to cold (maintenance in a 4°C environment for 4 weeks). This results in increased lipid turnover in brown adipose tissue (BAT), but not in white adipose tissue. The same response to cold was observed when BAT was excised from cold-acclimated rats and examined *in vitro*, as when BAT metabolism was examined *in vivo* (by following the fate of injected glucose-U-^{14}C). This suggested that the basic behaviour of the tissue itself changed as a result of acclimation to cold. The rôle of the SNS in mediating these changes was examined by studying tissue deprived of its sympathetic nerve supply. Two techniques of denervation were employed: immunosympathectomy (to produce a generalized partial sympathectomy) and surgical denervation (to produce selective denervation of one brown fat pad, leaving the other as an intact control). Both techniques impaired, but did not abolish, the changes observed when BAT was examined *in vivo*. This suggested that the sympathetic nervous system played a rôle in regulating some, but not all, of the metabolic response to cold. Although denervation impaired the metabolic changes of cold-acclimation when BAT was studied *in vivo*, it had no effect on the changes observed *in vitro*. Thus the SNS regulates BAT metabolism by controlling a process *in vivo*, such as blood flow, rather than by altering the basic metabolic behaviour of the tissue itself. The relation of these findings to adipose tissue metabolism in diabetes and starvation was discussed.

Dr. J. Birkbeck, Department of Paediatrics, University of British Columbia, Vancouver, spoke on *Modified Fat Diets in the Management of Juvenile Diabetes.* The potential value of altering the dietary lipid patterns in the prevention of degenerative cardiovascular disease has received considerable attention in recent years, culminating in the extensive National Diet-Heart Study in the U.S.A. Angiopathy is often a presenting feature in maturity-onset diabetes, whereas in juvenile diabetes, clinically evident angiopathy is rare for many years after onset. Accordingly, the child with diabetes should in theory be most likely to benefit from dietary modification, which might retard the development of angiopathy. The study which was presented was similar to that of Lloyd and collaborators in England, but was utilizing a more modest alteration in dietary lipid patterns which did not require the use of commercially unavailable foods. The early results showed a definite lowering of plasma cholesterol levels without serious disturbance to the subject's way of life or diabetic control. The results were reviewed in the light of other studies, and it was concluded that it is too early to assess the true effect of these diets on diabetic angiopathy.

Dr. C.H. Harley, University of Alberta Hospital, Edmonton, spoke on *Endogenous Hypertriglyceridaemia Demonstrated by Dietary Challenge in Patients with Premature Coronary Artery Disease.* Twenty-one patients under 50 years of age with proved coronary artery disease were studied for abnormalities of serum lipids, serum lipoproteins (particularly very low density lipoproteins), carbohydrate intolerance and carbohydrate inducibility by dietary challenge during a period of high carbohydrate feeding. Following baseline lipid and carbohydrate studies, the patients were placed on an 85% high carbohydrate liquid diet for seven days. Serum lipid and lipoproteins were obtained during and at the completion of the study. Nine male control subjects under 30 years of age were studied in a similar manner. Young controls were chosen deliberately to reduce the possibility of latent lipid or carbohydrate abnormalities. Results of the study revealed that mean baseline serum triglyceride values were significantly higher in the patient group than in the control. Similarly, the mean increase of serum triglycerides after seven days of high carbohydrate feeding was significantly higher in the patient group than in the control group. Eight of twenty-one patients showed elevated amounts of very low density lipoproteins even before the dietary study. Fourteen of twenty-one patients (66%) demonstrated the phenomenon of carbohydrate inducibility after seven days of high carbohydrate feeding. Four of twenty-one subjects showed abnormalities of oral glucose tolerance. It was concluded, therefore, that a large proportion of young patients with coronary artery disease convert carbohydrate into endogenous triglyceride to an abnormal degree and that a seven day period of high carbohydrate feeding is sufficient to demonstrate this abnormality. The practical value of a high carbohydrate liquid diet as a test for out-patients was suggested by this study.

The afternoon papers were followed by a general discussion on *Obesity and Diabetes,* moderated by Dr. C.K. Gorman, Department of Medicine, University of Toronto. Three questions were put up for discussion: 1. Is a fat person with glucose intolerance always a true diabetic — in other words, what is the exact definition of diabetes mellitus? 2. What do high immunoreactive insulin levels associated with obesity mean? Is this a failure of end-

organ response, or does it represent an increased production of insulin ? 3. Which is more dependent on insulin, fatty acid synthesis, or glucose uptake and metabolism ?

The discussion extended well beyond these topics. It was clear that while we recognized the close interrelationship between carbohydrate and lipid metabolism at the cell membrane, and inside the cell, this did not explain the higher incidence of diabetes in obese people, nor dit it explain the hyperinsulinaemia associated with obesity.

The evening session of October 27 consisted of a discussion about the future format of the diabetes workshops. It was moderated by Dr. H.G. Giese, and Dr. O.V. Sirek. It was unanimously agreed that no publication other than that of a brief report should be considered lest this could inhibit free exchange of ideas. For the same reason it was agreed that the number of participants should remain small and by invitation only. There was a consensus of opinon that the Third Diabetes Workshop in its present format is adequately fulfilling the needs of our scientific community.

The second morning's session was chaired by Dr. M. Verdy of the Hôtel-Dieu de Montréal.

The first speaker was Dr. A. Angel, Department of Medicine, University of Toronto. He spoke about the *Effect of Lipolytic Agents on ATP Metabolism in Adipose Tissue Cells.* Lipolytic hormones have been shown to lower the ATP content of adipose cells incubated *in vitro.* To examine this phenomenon in detail, isolated rat adipocytes were incubated (100 mg cells/ml KRB 5% albumin buffer) for 1 h with norepinephrine 1 μg/ml, ACTH 1 μg/ml or dibutyryl-cyclic-AMP (DBC) 2 mM. With norepinephrine or ACTH, the ATP content fell 40—70% below control, whereas DBC frequently reduced it to zero. The fall in ATP was dose-related to each lipolytic agent. Addition of glucose and insulin attenuated the ATP depression despite an increase in total lipolysis. Thus the rate or extent of lipolysis was not responsible for lowering cellular ATP. Pyruvate (10 mM) did not prevent the reduction of ATP by norepinephrine, thereby excluding substrate deficiency as the causative factor. Recovery of adipose cell ATP levels was observed after reincubation of cells previously exposed to norepinephrine. The effects of DBC were irreversible. Extracellular free fatty acid accumulation could not be implicated because ATP levels were maintained in cells incubated in media containing 4 mM Na-oleate (FFA/albumin molar ratio 7.5/1). On incubating cells in fatty-acid-saturated media, the sensitivity to lipolytic agents fell. However, under these conditions cellular ATP was reduced when total lipolysis was far below that usually observed. These data implicate intracellular accumulation of FFA in lowering adipose cell ATP.

Dr. M.L. Halperin, Department of Medicine, University of Toronto, spoke on the *Control of Fatty Acid Synthesis in White Adipose Tissue.* The rate of glucose metabolism in white adipose tissue seems to be controlled under physiological circumstances by the rate of glucose entry into the cell (augmented by the availability of insulin). Regulation of the direction of flow of glucose carbons to either fatty acid, glyceride-glycerol, lactate or CO_2 has not been elucidated. The cytoplasmic NADH/NAD occupies a critical position in glucose metabolism, i.e. dihydroxyacetone phosphate → L-glycerol-3-phosphate and pyruvate → lactate. Both these reactions are favoured by a high cytoplasmic NADH level, which would therefore result in diversion of the glucose carbon from the fatty acid synthesis pathway. On conversion of glucose to fatty acid, NADH is produced in excess of that needed for fatty acid, L-glycerol-3-phosphate and lactate productions. Cytoplasmic NADH must be removed primarily with oxygen. Mitochondrial transport of NADH was studied polarographically utilizing white adipose tissue mitochondria. Results indicate that these mitochondria have a permeability barrier to NADH. The other potential pathways of NADH transport-L-glycerol-3-phosphate shuttle and malate-oxaloacetate shuttle (Borst, 1963) — were studied and found to have very low activity in this tissue, as L-glycerol-3-phosphate is oxidized at an extremely slow rate in the first case, and the intramitochondrial glutamic-oxaloacetic transaminase activity seems to limit the second case. The cytoplasmic NADH concentration could be readily modified and therefore influence the rate of fatty acid synthesis from glucose.

Dr. N. Forbath, Department of Medicine, University of Toronto, presented a paper on *Metabolic Interrelations of Glucose and Galactose in unanaesthetized Normal and Diabetic Dogs.* Uniformly labelled ^{14}C-glucose infusion was given to normal and pancreatectomized dogs. Plasma glucose and lactate specific activities were measured by appropriate methods. 14—28% of plasma lactate was found to originate from circulating glucose in normal and 11—18% in diabetic dogs. This fraction was markedly increased in normal but not significantly in diabetic dogs during a two-hour infusion of a 6.7—11 mg/kg/min glucose load.

Uniformly labelled ^{14}C-L(+)-lactate and glucose-6-^{3}H were infused simultaneously for 5 h in normal and pancreatectomized dogs. Glucose turnover rate and the incorporation of lactate carbon into glucose were calculated. 41—49% of the utilized lactate carbon was incorporated into plasma glucose. The recycling rate of glucose carbon via lactate was estimated to be 3—8% of the glucose turnover rate in the normal fasting dog. The absolute rate of gluconeogenesis from lactate was found to be increased in diabetes.

The afternoon session was chaired by Dr. Anna Sirek, Division of Teaching Laboratories, Faculty of Medicine, University of Toronto.

The first paper was presented by Dr. J. Martin, Research Institute of the Hospital for Sick Children, Toronto. He spoke on *Metabolic Changes in Adipose Tissue of Rats Bearing a Growth Hormone Secreting Tumour.* Endogenous growth hormone was elevated in rats inoculated with a transplantable pituitary tumour (MtTW15). Coincident with the rise of circulating GH, serum free fatty acid concentrations also rose. An increased lipolysis and a decreased glucose utilization by adipose tissue *in vitro* could not be corrected by the addition of insulin when the animal had been exposed to high levels of growth hormone for more than seven weeks. These metabolic changes in adipose tissue developed in three stages as shown by studies done on isolated fat cells taken from animals at different periods after the implantation of the tumour. At the beginning there was an increased glucose utilization (oxidation to CO_2 and incorporation into triglycerides) both in the presence and absence of insulin. The following period was characterized by a decreased glucose utilization which was still correctible by the addition of insulin. In the last stage insulin resistance became evident. The combined effect of decreased lipogenesis and increased lipolysis finally resulted in the almost complete depletion of fat depots observed in these tumour-bearing rats. In spite of these "diabetic" changes observed *in vitro,* the tumour-bearing rats did not become clinically diabetic.

Dr. Martin's presentation was followed by a number of short preliminary communications.

Dr. P.P. Foà spoke on *Adipose Tissue Lipolysis in Golden Hamsters with Chronic Hypoglycaemia and Hyperinsulinaemia Due to a Transplantable Islet Cell Tumour.* Golden hamsters bearing a transplantable islet cell tumor had high plasma immunoreactive insulin (IRI) and low blood glucose concentrations. No correlation was found between blood glucose, plasma IRI and plasma free fatty acids (FFA). *In vitro,* the epididymal adipose tissue of tumour-bearing hamsters released more glycerol than that of control hamsters, but since the re-esterification of FFA

was also increased, the net FFA release was lower than that of normal animals. At a glucose concentration of 100 mg%, glucose uptake and glycerol release were higher and the FFA release was lower than at a glucose concentration of 25 mg%. Prostaglandin E_1, at concentrations of 1 or 5 μg/ml, reduced glycerol release by the adipose tissue of normal fasted or fed hamsters, but had no effect on the glycerol release of fasted, hypoglycaemic tumour-bearing animals.

Dr. O.V. Sirek, Department of Physiology, University of Toronto, spoke on the *Effect of Dihydroergotamine on Lipolysis Caused by Growth Hormone, Prolactin and Placental Lactogen in Dogs*. The initial reduction in the concentration of plasma free fatty acids (FFA) which occurs following a single intravenous injection of growth hormone (GH) was abolished and replaced by an early and steep rise in FFA when dogs were pretreated with dihydroergotamine (DHE). Phentolamine, also an adrenergic blocking agent, had no such effect. DHE altered the lipolytic effect of human placental lactogen in a fashion similar to that of bovine GH, but had no effect on lipolysis produced by bovine prolactin. The lipolytic effects of ACTH or TSH were not affected either. It was suggested that DHE could serve as a tool in elucidating the details of the lipolytic action of GH. As a first step in this direction Dr. N. Hotta, postdoctoral fellow from the University of Nagoya, Japan, studied in Dr. Sirek's laboratory the *Effect of Dihydroergotamine on the Isolated Fat Cell*. The drug was lipolytic *in vitro* and the effect was augmented in the presence of theophylline. Beta adrenergic blocking agents abolished the lipolytic effect of DHE. Phentolamine was ineffective when tested in the isolated fat cell system, although it is known to be lipolytic *in vivo*. These results indicate that DHE has 1. a direct effect on adipose tissue cells and 2. beta adrenergic receptors, adenyl cyclase, and CAMP are involved in DHE-induced lipolysis. The relationship to GH remains to be elucidated.

Dr. C.K. Gorman spoke briefly on *Insulin Fat Atrophy — Possible Cause and Possible Treatment*. The cause of this sometimes disfiguring condition has not been identified. A very similar deformity developed in one patient who received subcutaneous glucagon injections for six months. Since insulin is lipogenic, and glucagon is lipolytic, and since most insulin preparations contain small amounts of glucagon, the suggestion was made that the insulin-induced fat atrophy may in fact be the result of glucagon contamination in the insulin.

The final paper was presented by Dr. B.J. Kaufman, Department of Medicine, University of Manitoba, Winnipeg. He spoke on *Serum Lipid Assays Using Fingertip Blood Samples*. The current interest in population surveys for metabolic disorders has made the development of techniques for the rapid determination of cholesterol and triglycerides desirable. In order to avoid the difficulty of venepuncture and the inconvenience of separation of plasma, a method of assaying these lipids, using fingertip blood samples was devised. In 43 fasting subjects mean ± S.D. values for cholesterol were: venous plasma 191 ± 32 mg%, venous blood 179 ± 20 mg%, capillary blood 178 ± 20 mg% and capillary blood/venous plasma ratio 0.94 ± 0.07. The corresponding values for triglycerides were: 152 ±70, 93 ±43, 98 ±43, 0.66 ± 0.07. In 15 postprandial subjects the capillary blood/venous plasma ratios were: 0.99 ± 0.06 for cholesterol and 0.62 ± 0.05 for triglycerides. In a pilot study of 1000 adults using random fingertip blood samples, cholesterol values exceeded 200 mg% in 10%, and 220 mg% in 5% of the subjects; triglyceride values exceeded 200 mg% in 10% and 250 mg% in 5% of the subjects. During a 1969 diabetes detection survey, which is currently proceeding, random postprandial fingertip blood samples were also obtained for cholesterol and triglyceride analyses. Up to September 15th, 1969, about 5000 persons had blood taken for lipid analyses and 229 subjects whose serum cholesterol and/or triglyceride values exceeded "cut-off" levels of 220 mg% and 250 mg% respectively, were recalled and fasting venous blood samples were obtained. Also, follow-up fasting venous samples were obtained from 184 persons with serum lipid values less than the "cut-off" levels. The reliability of these "cut-off" levels in predicting high fasting venous plasma cholesterol and/or triglyceride levels seems reasonably good.

Dr. O.V. Sirek in his concluding remarks, as organizer and participant, thanked Dr. M. Blake, Hoechst Pharmaceuticals, Montreal, for his tireless efforts as Executive Secretary. Words of sincere appreciation went to Dr. H. G. Giese for his steady interest and support; and last but not least to Dr. C.K. Gorman for help in organizing the scientific programme.

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