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REPORTS FROM MEETINGS

The Second Canadian Workshop on Diabetes

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The Second Canadian Workshop on Diabetes, with 18 participants, took place on October 28 and 29, 1968, at 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 this year's Honorary President, Dr. A.L. CHUTE, Dean of the Faculty of Medicine, University of Toronto. Dean CHUTE chaired the morning session of October 28.

The first paper was presented by Dr. R.E. HAIST, Department of Physiology, University of Toronto, on *Bioassays of Insulin*. The types of bioassay were reviewed, and the sensitivity of the various assays compared. The fact that an activity is being measured by bioassay, and that there is a possibility of different effects of insulin on different activities was discussed. The effects of differences in the degradation of insulin in standard and unknown solutions was noted. A 5% gelatin solution, serum, and acid-alcohol-treated serum were effective in preventing most of the insulin degradation. Using the mouse hemidiaphragm preparation, standard doses of insulin were found to have greater effects on the glycogen of diaphragm in the presence of serum and treated-serum than in the presence of a balanced salts-gelatin solution. When the hemidiaphragm bioassay was used with standards made up in balanced salts-gelatin solutions, the measured recovery of added insulin was greater than that added. When the hemidiaphragm bioassay was used with standards made up in treated-serum from depancreatized animals, the measured recovery of added insulin was similar to the amount added. When unknown solutions were sufficiently potent and were diluted with a balanced salts-gelatin solution (the same as that used to dilute the standard insulin), the insulin values were similar to those obtained with the BERSON-YALOW immunoassay. The conclusion was that when hemidiaphragms were incubated with standard and unknown insulins which were present in highly similar media, then any insulin added to the unknown could be recovered quantitatively. The assumption was that the amount of endogenous insulin would be estimated quantitatively also. The importance of bioassay procedures was discussed.

Dr. C.C. YIP, Banting and Best Department of Medical Research, University of Toronto, next presented a paper on *Recent Studies of the Biosynthesis of Insulin*. Using slices of foetal bovine pancreas incubated with ^{3}H-leucine, the synthesis of a single-chain insulin precursor, proinsulin, was demonstrated. This precursor was: i) immunoreactive towards guinea pig anti-bovine insulin serum, and ii) converted to a substance similar in size to bovine insulin by controlled trypsin hydrolysis. The trypsinized substance was broken by sulphitolysis into two subunits, which were identical in gel filtration. to the A and B chains of bovine insulin. A single-chain and a double-chain proinsulin have also been obtained from commercial preparations of bovine insulin. The single-chain proinsulin contained 77 amino residues, 26 residues more than that present in insulin, with the extra amino acid residues linking the carboxyl terminus of the B chain with the amino terminus of the A chain of insulin. In the double-chain proinsulin, the additional polypeptide less one arginyl residue was attached to the carboxyl terminus of the B chain. A proinsulin specific antiserum has been obtained by absorption of guinea pig anti-proinsulin serum with a solid immunosorbant of Sephadex-insulin. This specific antiserum reacted only with proinsiuln but not insulin in a double-antibody immunoassay system, and exhibited positive passive cutaneous anaphylaxis reactions only with proinsulin and not with insulin. Preliminary experiments using this specific antiserum showed the presence of proinsulin in native calf and human sera.

Dr. J. LOGOTHETOPOULOS, Banting and Best Department of Medical Research, University of Toronto, spoke on *Aspects of Cell Regeneration of the Islets of Langerhans*. Severe hyperglycaemia was induced and maintained in mice by intraperitoneal injections of globulins from guinea pigs immunized with beef insulin. Radioautography following injection of ^{3}H-thymidine, or arresting of mitoses at metaphase with Colcemid, was used to estimate new production of beta cells. A wave of mitotic activity three to ten times greater than that of controls appeared after a latent period of twenty-four hours. Dividing beta cells were randomly located throughout the islets. No evidence of duct-cell mitotic activity was obtained. Food restriction and injection of actinomycin D markedly decreased the stimulated mitotic rate. A short treatment with tolbutamide did not affect the mitotic rate. The radioactive (proportion of labelled nuclei) and mitotic (proportion of metaphases) indices of beta cells during a second stimulation following a normoglycaemic period of two weeks were less than half of those found in mice during the first stimulation. Young beta cells which arose from recent divisions were found to be much less responsive to a second mitotic stimulus than "old" beta cells at the end of a long intermitotic phase.

In another series of experiments mice were made diabetic with alloxan or streptozotocin. The mitotic activity of the surviving beta cells was estimated at various periods after the induction of diabetes, using radioautography. A mitotic wave was observed during the first two weeks. This activity subsided thereafter to levels below the control values. No evidence of formation of beta cells from acinar or duct cells was found. Alpha and acinar cells did not show a mitotic rise after alloxanization, but duct cells responded with a transient increase in mitotic divisions.

The afternoon session was chaired by Dr. G.E. JORON, Department of Medicine, McGill University, Montreal.

Dr. D.L. WILANSKY, Jewish General Hospital, Montreal, presented a paper entitled *Early Latent Diabetes: Further Observations*. He reviewed the British Diabetic Association classification of the preclinical phases of diabetes mellitus, inculding definitions of the terms prediabetes, latent diabetes, and diabetes. Observations were presented to indicate that individuals with a borderline abnormality in their cortisone glucose tolerance test (G.T.T.) had maximal abnormalities in lipid metabolism and a diminished insulogenic index with no lag. From this group of subjects, approximately 12% of a group of 60 developed diabetes during a three-year period of observation. The findings suggested that early latent diabetes mellitus is a distinct entity. In addition, the course of latent diabetes was described, and the result of a pilot study concerning the effect of a six week course of phenformin in modifying and reducing the onset of diabetes was presented.

The next paper was given by Dr. J.B.R. MCKENDRY, Metabolism Department of the Ottawa Civic Hospital. His topic was *Idiopathic Intermittent Oedema of Women — a Manifestation of Diabetes*. In a study of 111 women with the syndrome of idiopathic intermittent oedema, a total of 50 were diabetic, or proved to be diabetic on initial glucose screening. A further 2 became diabetic during a

follow-up period, 5 others had an abnormal G.T.T. with a peak exceeding 160 mg%, and a further 5 had an abnormal cortisone G.T.T. In 35 the oedema had preceded the diagnosis of diabetes, in 5 the oedema and diabetes were diagnosed within the same year, and in 10 the diabetes preceded the oedema.

The average age of onset of the oedema in those known to be diabetic was 36 years, and for those not yet diabetic 32 years. The age at which idiopathic intermittent oedema was diagnosed was 44 for the diabetics, and 39 years for those not yet diabetics.

Fifty-one of the 111 were non-diabetic by G.T.T., and the status of 5 unknown. Of 45 women not yet diabetic for whom full history was available, 42 had a history of such prediabetic stigmata as big babies, diabetes, arteriolosclerotic heart disease or obesity, and 39 had a personal history of such prediabetic stigmata as big babies, reactive hypoglycaemia, postural hypertension or peripheral neuritis. Sixteen of 36 who had been pregnant gave a history of one or more abortions.

The data were interpreted as support for the conclusion that idiopathic intermittent oedema syndrome bears a remarkable relationship to diabetes and may, indeed, be a manifestation of diabetes in women.

The last paper of the afternoon session was presented by Dr. H.R. HAUSLER, Department of Ophthalmology, University of Toronto. He discussed *Newer Concepts of the Pathology and Treatment of Diabetic Retinopathy.* He pointed out that a diabetic is almost 15 times as likely to become blind from retinopathy as a non-diabetic from all causes of blindness. Diabetic retinopathy ranks now among the main causes of blindness in the Western World, being responsible for about 15 to 20% of the registered blind. Fluorescein injection studies show that in the diabetic retina a functional microangiopathy exists long before the clinical-morphological microangiopathy becomes apparent. The microangiopathy manifests itself first in the basement membrane of the capillaries, possibly due to metabolic changes in endothelial cells and/or the surrounding neural and neuroglial tissue. The role of the intramural pericyte is still controversial and was discussed. Localized areas of retinal ischaemia may either precede, or be secondary to aneurysm formation and neovascularization. The possible involvement of insulin antibodies was mentioned. The therapy of diabetic retinopathy was considered to be largely unspecific and ineffective. New trans-sphenoidal stereotaxic thermal procedures were mentioned. The lack of controlled studies in most surgical procedures was pointed out. The role of therapeutic and prophylactic photocoagulation of diabetic retinae was discussed. In conclusion, the needs for adoption of a uniform classification, uniform methods of recording, standard follow-up periods, and controlled studies in the evaluation of the different forms of therapy were stressed.

The evening session of October 28 consisted of a discussion about the future of the diabetes workshops. It was moderated by Dr. H.G. GIESE, Hoechst Pharmaceuticals, Montreal, and Dr. O.V. SIREK, Department of Physiology, University of Toronto. It was unanimously agreed that the workshops served a useful purpose and it was recommended that future meetings should include only a few formal presentations on a selected topic. This would then leave plenty of time for free discussion which is considered to be the main asset of a small workshop.

The second morning's session was chaired by Dr. W. T.W. CLARKE, Department of Therapeutics, University of Toronto.

The first speaker was Dr. CALVIN EZRIN, Departments of Pathology and Medicine, University of Toronto. He spoke about *The Cytophysiology of the Human Adenohypophysis, with Particular Reference to the Prolactin Cell.* He pointed out that the human adenohypophysis is known to produce at least 7 hormones, viz: FSH, LH, TSH, ACTH, MSH, GH, and Prolactin. In recent years, special staining procedures performed on glands from selected conditions that were associated with altered secretion of these hormones have helped to define their separate cells of origin. These techniques have defined seven different cells responsible for the production of the seven hormones of the adenohypophysis. The acidophil-basophil-chromophobe classification that was originally employed in describing the morphology of the pituitary is still useful as a means of comparing the results of newer staining procedures. Modifications of the periodic acid-Schiff (PAS) procedure demonstrate basophils and related chromophobes as cells with red or purple-staining granules. Four sub-types of basophils have now been defined:

1. The beta-1 cell is the most obvious basophil in most glands; it is usually large and angular, and it always stains red with PAS. It undergoes the Crooke's hyaline change after prolonged steroid therapy or in Cushing's syndrome. It is probably the source of MSH.

2. The beta-2 cell has more affinity for aldehyde thionin than for PAS, and ultimately stains blue-black in the aldehyde thionin-PAS technique. Because it undergoes selective hypertrophy in hypothroidism and regression in hyperthyroidism (from any cause), it is considered to be the source of TSH.

3. The delta-1 cell is small, oval with coarse aldehyde thionin-positive granulation. It is present in large numbers only in adults. It is probably the source of FSH.

4. The delta-2 cell is also aldehyde thionin-positive, but its granules are finer than those in the delta-1 cell. Because it selectively takes up fluorescein-labelled anti-human chorionic gonadotrophin (HCG), it is felt to be the source of LH — which cross-reacts well with HCG.

Two sub-types of acidophils have been defined by combinations of acid stains, usually employing Orange G and some other procedure. The growth hormone cell is the most numerous acidophil; it is found at all ages and in both sexes. Its granules have more affinity for Orange G than the other dyes, and are smaller than those of the second type of acidophil. The prolactin cell is erythrosinophilic and carmoisinophilic. It is usually larger than the growth hormone cell and contains coarser granules. It is found in large numbers in the last trimester of pregnancy and during lactation, but is rarely seen in other glands. In one case of prostatic carcinoma treated with large doses of estrogen, large numbers of prolactin cells were found. The ACTH cell is thought to be chromophobe, without much affinity for either acid or basic dyes. There was no characteristic pathology of any of these cells found in diabetics.

The separate identity of growth hormone and prolactin cells seems obvious morphologically. The failure of extraction methods to isolate human prolactin separate from growth hormone may be explained by the use of pooled pituitaries containing few, if any, glands from cases of pregnancy or lactation, the only source likely to yield significant quantities of this hormone.

Two papers on *The Acute Effects of Growth Hormone on Plasma Free Amino and Free Fatty Acids in Dogs* were given by Dr. A. SIREK and Dr. O.V. SIREK, both of the Department of Physiology, University of Toronto. The acute reduction in the concentration of plasma free amino acids was observed in normal, hypophysectomized, pancreatectomized, and doubly-operated Houssay dogs following a single injection of bovine growth hormone. The reduction obtained after growth hormone in diabetic animals was completely abolished by administering dihydroergotamine (DHE) or 2-brom-d-lysergic acid diethylamide (BOL-148). Pharmacological blockade of the adrenergic and serotonergic systems partially counteracted the growth hormone effect in normal and hypophysectomized dogs; complete suppression was achieved only in combination with puromycin. These results were

taken to indicate that the reduction in the concentration of plasma free amino acids following a single injection of growth hormone had at least two components. It was suggested that one was protein anabolic in nature, and the other involved hepatic gluconeogenesis caused chiefly by serotonin.

The initial reduction in the concentration of plasma free fatty acids (FFA) observed following a single i.v. injection of bovine growth hormone was abolished and replaced by an abrupt and steep rise when hypophysectomized dogs were pretreated with DHE. The acute hyperlipaemic response could be suppressed with puromycin. Neither phentolamine (alpha adrenergic blocking agent) nor Kö 592 (beta adrenergic blocking agent) had the same effect as DHE. The serotonin antagonist BOL-148 was also ineffective. The data indicated that DHE, by a mechanism probably not involving adrenergic receptors, altered the lipolytic response to a single dose of growth hormone.

The final paper of the morning session was presented by Dr. C.K. GORMAN, Department of Medicine, University of Toronto. He discussed *The Influence of Insulin on the Removal of Sodium Octanoate from the Incubation Medium by Rat Liver Slices.* Glucagon has been reported to stimulate FFA removal from the perfusate by the isolated rat liver, and to have the same effect on the removal of sodium octanoate by rat liver slices *in vitro*. Indirect evidence made it very improbable that this effect was due to insulin contamination. Experiments were carried out to investigate this problem more directly. Liver slices from fed and fasted rats were incubated in Krebs-Ringer bicarbonate buffer containing 2.0 μEq/ml of sodium octanoate and 5% bovine serum albumin. Insulin, in concentrations ranging from 0.03 to 3.0 mU/ml, was added to some of the incubates, and the removal of FFA from the medium was measured 2 h later. Examination of the results showed that the higher the insulin concentration, the lesser the removal of the sodium octanoate. The effect was often highly significant at insulin concentrations of 0.3 mU/ml with the tissue from fed rats, and at 3.0 mU/ml with tissue from fasted rats. The significance of these results, in relation to the normal concentration of insulin in the portal vein, and possible explanations of the effect, were discussed.

Dr. J. BRUNET, Department of Medicine, Laval University, Quebec City, chaired the afternoon session.

The first paper was given by Dr. B. ISSEKUTZ, Dalhousie University, Halifax. He discussed *Exercise Metabolism in Diabetes.* Glucose-^{14}C or palmitate-^{14}C, respectively, were infused at a constant rate into normal and pancreatectomized dogs running on the treadmill for 3—4 h. Hepatic glucose output and relase of FFA, as well as the rate of oxidation of plasma glucose or that of FFA, were calculated. Exercise increased the metabolic rate some six-fold. In control dogs the hepatic glucose output rose 2.5-fold, and it was immediately converted to CO_2 at a rate equal to the rate of hepatic output. In diabetic dogs the rate of oxidation of plasma glucose was less than one half of that found in controls. The hepatic glucose output was far in excess of the rate of oxidation. The plasma glucose level of diabetic dogs rose during exercise, whereas it decreased in the controls. When glucose was infused into normal dogs to maintain the blood sugar concentration at a level of about 100 mg%, the rate of oxidation increased to a value more than eight times from that found in pancreatectomized dogs at 400 mg% plasma glucose. It was concluded that the utilization of glucose remained severely impaired during exercise. Under any circumstances FFA represented the major energy source for exercise in control as well as in diabetic dogs. When in this latter group the rate of release of FFA was inhibited by nicotinic acid, the contribution of glucose to the exhaled CO_2 (about 10%) failed to increase, but the hepatic glucose output and the blood glucose level decreased during exercise. In diabetes the extremely high FFA level seems to play a role in the enhanced hepatic glucose output, but it cannot be made responsible for the impaired glucose oxidation of the working muscles.

Dr. J.A. MOORHOUSE, University of Manitoba, Winnipeg, presented results of *Quantitative Studies of Glucose and Cortisol Metabolism in Man.* Estimations of endogenous glucose turnover were done in healthy subjects between 9.00 a.m. and 12.00 noon by a) estimation of the blood glucose specific activity at zero time following a single injection of glucose-U-^{14}C (8.8 ± S.E. 0.6 g/h), and b) by measurement of the specific activity of the glucose pool at equilibrium during the continuous infusion of glucose-U-^{14}C following a priming dose (6.6 ± S.E. 0.2 g/h). The higher values obtained by the single injection technique are believed to be due to incomplete mixing of the isotope, and the continuous infusion technqiue is believed to be the preferable method. However, when studies in diabetic subjects were undertaken during the morning hours a marked decline in the blood glucose level was observed, and achievement of a steady state was not possible. Subsequent studies demonstrated this to be due to a diurnal variation in the level of blood glucose in diabetics (Clin. Sci. **32**, 111, 1967). During these studies it was noted that the diurnal change was in phase with the serum cortisol level, and that the serum cortisol levels were higher in diabetic than in healthy subjects. Follow-up studies presently in progress indicate: 1. In over-weight diabetic subjects aged 40 to 65, compared with non-diabetic subjects of similar weight and age, the 9.00 a.m. serum cortisol level was higher (22.9 μg/100 ml ± S.E. 1.3. and 17.2 ± 1.7 $p < 0.02$), the serum cortisol-binding globulin level was lower (31 mg% ± S.E. 2.4 and 36 ± 2.7, $p < 0.01$), and the urine free cortisol was the same (218 μg ± S.E. 25 and 194 ± S.E. 23, $p < 0.3$). 2. Glucose turnover was higher between 6.00 and 9.00 a.m. than between 6.00 and 9.00 p.m. in both healthy and diabetic subjects, was higher at both times in diabetes than in health, and was linearly proportional to the blood glucose level.

Dr. O.V. SIREK in his concluding remarks thanked Dr. M. Blake, Hoechst Pharmaceuticals, Montreal, for his tireless efforts as Executive Secretary. Words of sincere appreciation went to Dr. C.K. GORMAN for his help in organizing the Workshop, and last but not least, to Dr. H.G. GIESE for his interest and support. Dr. GIESE closed the Meeting by announcing that the next Workshop will be held again in October 1969.

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