Special Article

Fifth Congress of the International Diabetes Federation, Toronto, 1964

Part I of a Two-part Review

R. E. Haist, M.D., Toronto

The Fifth Congress of the International Diabetes Federation was held in Toronto, Canada, July 20-24, 1964. Over two thousand people from forty-five different countries were present. These included most of the outstanding investigators and many clinicians concerned with diabetes and related problems. The opening meetings on the first morning of the Congress were presided over by Dr. F. C. Jeanneret, Chancellor of the University of Toronto, and members were welcomed by the Lieutenant Governor of the Province of Ontario, representatives of the federal, provincial and municipal governments, the President of The Canadian Diabetic Association and the Chancellor. The opening meeting concluded with the Presidential Address by Dr. Howard F. Root of Boston.

Among the special events was an evening Convocation at the University of Toronto at which honorary degrees were conferred on Dr. Joseph P. Hoet of Belgium, Dr. R. D. Lawrence of England and Dr. Randall Sprague of the U.S.A. Dr. Sprague addressed Convocation, telling of some of his experiences in diabetes therapy as a patient and as a physician dating from the early days of insulin. Receptions, dinners, local and special tours were provided in order to make the visit of the members more enjoyable.

It was a great sorrow for all that Dr. C. H. Best, one of the co-discoverers of insulin, was not able to be present because of illness, especially since he had played such a large part in organizing the Congress.

The main purpose of the meetings was to present and discuss the research findings in the areas concerned with diabetes and in the lay or general sessions, to deal with social and other problems of diabetics and to present some of the newer scientific advances to them. The scientific program featured eleven symposia, dealing with special topics, the papers being presented by invited speakers. The free communications were of high quality also, there having been a selection of 138 papers from the 359 contributed.

The symposium papers edited by B. S. Leibel and G. A. Wrenshall, the joint chairmen of the Scientific Program and Publication Committee, will be published by *Excerpta Medica* (Amsterdam) as a volume entitled *On the Nature and Treatment of Diabetes,* which should be available in the early part of 1965.

From the Department of Physiology, University of Toronto. Dr. Haist was acting chairman, Local Organizing Council, Fifth Congress, International Diabetes Federation.

THE ISLET CELLS

In the first symposium on the *Islet Cells, Paul E. Lacy* dealt with *Studies by electron microscopy of secretory processes of islet cells.* He showed that, under the electron microscope, beta cells, alpha cells with three sub-types, C cells, D cells and X cells could be distinguished. Despite this, so far, only two hormones have been isolated from the islets. A marked species variation in the structure of the beta granules was observed. The secretory process in the rat appeared to be a simple extrusion of the granules from the beta cells, the process being similar when the cell was stimulated with glucose or tolbutamide. Granules seemed to reform within the ergastoplasm. The administration of anti-insulin serum to the rat led to degranulation of beta cells and evidence of inflammatory changes.

In the next paper *R. E. Haist* reviewed *The effects of changes in stimulation on the structure and function of islet cells.* He pointed out that the major functions of the beta cells are to synthesize, store and secrete insulin and to increase in number when the secretion of existing cells is inadequate. The structural changes related to high levels of activity are increase in nuclear size and in the prominence and complexity of the Golgi apparatus, and reduction in the granules of beta cells. If the stimulation is continued there may be glycogenic infiltration of beta cells and hydropic change. Continued stimulation leads also to proliferation of beta cells and, in some species, the mass of islet-producing tissue is increased, and the potentiality for function enhanced. Changes in granulation and insulin content are dependent upon the time and rate relationships between synthesis and secretion. Insulin secretion is increased by glucose stimulation, by tolbutamide and by a number of factors causing hyperglycemia. The glucose supply to the pancreas may be important in relation to insulin synthesis.

Arnold Lazarow dealt with the *Functional characterization and metabolism of islet cells.* Goosefish islet tissue, incubated in vitro, incorporates radioactive amino acids into protein. When this tissue was incubated with radioactive glucose the label was incorporated into the goosefish insulin. Increase in the concentration of glucose reduced the incorporation of radioactive amino acids into acid-alcohol soluble protein but enhanced the incorporation of radioactive glucose into such protein. The administration of tolbutamide inhibited glucose incorporation but not leucine-H-3 incorporation into the acid-alcohol soluble protein. He suggested that the A and B chains

of insulin may be synthesized separately and then joined by oxidizing the sulfydryl groups to disulfide bonds. His findings support the idea that insulin is synthesized in the microsomes and subsequently transferred to the granule. An additional interesting finding was that the islet tissue of the toadfish was freely permeable to glucose. Lazarow and his associates also studied the enzymes of the islet cells, and the insulin content of microdissected islets. They found that after alloxan, the insulin content of the rat islet showed little change for twenty-four hours but fell to less than 5 per cent of the control at forty-eight hours. With other evidence it was suggested that the beta cell membrane may be a prime site of action of alloxan in producing diabetes, the alloxan presumably combining with dithiol groups of the membrane.

In the discussion of this section of the symposium, *S. Falkmer* added some observations on cyclostomes and teleost fish. *S. S. Lazarus,* because of certain electron microscopic changes, suggested the possibility that the osmophilic granule of the beta cell is a storage form, and that lytic enzymes may alter the storage material to a soluble product which is then liberated. *B. W. Volk* questioned the justification for giving the various cell types different names and wondered whether or not they might represent dying or degenerating beta cells, a suggestion that was opposed by P. E. Lacy.

G. Hultquist in a paper entitled *Ocular transplants of islet tissue in diabetic and nondiabetic rats,* described the transplantation of small pieces of atrophied tissue from duct-ligated pancreas into the anterior chamber of the eye. It was shown that the cells of the transplants were morphologically similar to the normal alpha and beta cells. In the transplants into hypophysectomized rats, islet tissue was evident, and in controls and in diabetic rats, hydropic changes in the beta cells were seen under some circumstances. Nuclear enlargement and hydropic changes seemed to be related to the concentration of blood glucose. Hyperglycemia increased the beta cell activity, thus providing evidence for a direct stimulation of beta cells by glucose. The conclusion was that the grafts could function and that exchanges between the graft and the host could be carried out.

Bo Hellman dealt with *The microchemical analyses of pancreatic beta cells isolated from mammals.* He described a method for obtaining isolated islet cells from fresh pancreas by free hand dissection and, using this, isolated fresh islets from obese hyperglycemic mice. Enzyme activities of the isolated islets were determined and evidence obtained for the presence of two different acid phosphatases, sulfydryl dependent ATPase and 5-nucleotidase. In cortisone-treated rats the 5-nucleotidase activity increased and ATP breakdown was reduced. Alloxan occasioned rapid reduction of ATPase and acid phosphatases. Studies of the isolated granules indicated that they represent insulin storage sites.

The next paper, by *G. H. Dixon,* dealt with *The recombination of insulin A and B chains, hybrid insulins and synthetic insulins.* He showed that highly purified A and B chains were devoid of insulin-like activity but that a mixture of the two chains treated with thiol-2-mercapto-ethanol gave definite insulin activity as shown by both the mouse convulsion and hemidiaphragm assays. This activity was neutralizable by guinea pig anti-ox insulin.

Studies of cod and ox insulin showed that the A chains were different but the B chains were more similar in re-synthesized insulin containing cod A chain or in the native insulin containing cod A chain, neutralization by guinea pig anti-ox insulin antibody was poor whereas if ox A chain was present in the molecule neutralization was good. The ox A chain seemed to be the primary antibody-binding site of the molecule. Insulin activity was obtained after synthesis using synthetic A and B chains.

H. Zahn, in his discussion of this paper, noted a slight but definite insulin-like activity of the synthetic A chain alone. *S. Wilson* presented evidence indicating that there may be insulin formation in non-vertebrate species such as the star-fish. He noted, in relation to Dr. Dixon's paper that the ox A-cod B hybrid was neutralized by anti-ox-insulin serum but not by anti-cod-insulin serum, whereas with cod A-ox B hybrid the reverse was true, the explanation being that the antigenic site lies on the A chain. Of interest here was the finding that the insulin from a low order teleost, the bowfin, was neutralized by both the anti-cod and anti-ox insulin serum, suggesting that the bowfin insulin may have two antibody-combining sites.

METABOLISM OF SELECTED TISSUES

In *Symposium Number 2,* on *Features of metabolism characteristic of selected tissues,* the first paper was given by *M. E. Krahl, J. C. Penhos, and A. Kraemer* and dealt with *The effects of insulin on the protein metabolism of liver.* They showed that the ability of the liver to synthesize certain proteins could be affected by insulin. In diabetes, the enzymes concerned with the synthesis of glycogen, fatty acids, and protein tended to be reduced in concentration whereas those involved in gluconeogenesis tended to be increased. The incorporation of carbon-labelled leucine into protein in liver-slices decreased as the severity of the diabetes increased and in the microsomal fraction from livers of diabetic rats there was impaired incorporation of amino acids into protein. The changes were reversed by the injection of insulin.

The next paper by *K. L. Manchester* dealt with *Insulin and protein metabolism in muscle.* Insulin has a stimulating effect on the incorporation of labelled amino acids into protein in diaphragm, heart, fat-pad, adrenal, bone marrow, mammary gland, cell cultures and chicken embryo heart. This effect apparently is not secondary to the utilization of carbohydrate and does not seem to be due to changes in the transport of amino acids. The action however is limited to tissue preparations and disappears when the cells are destroyed.

In discussion of these two papers *G. F. Cahill, Jr.,* stated that, in fish, insulin has little hypoglycemic effect but markedly enhances the incorporation of amino acids into protein. Also, the amino acid, leucine, stimulates the beta cells of the fish islets. *H. D. Soeling* mentioned that in isolated, perfused rat livers the uptake of amino acids can be increased by biguanides and quoted Lipmann as showing that peripheral tissues of eviscerated hepatectomized dogs took up amino acids under the influence of biguanides. *Jean Manery* mentioned a marked stimulating effect of insulin on the oxidation of lactic acid by frog muscle and *E. Copp* brought up the effect of variations in the levels of sodium or potassium on the activity of insulin.

The paper by *Alberto Sols* dealt with *The regulation of liver glucokinase and muscle hexokinase.* The phosphorylation of glucose was reported to be carried out by hexokinase and a

unique glucokinase. The unique glucokinase, found in liver but not in muscle, was induced by insulin, adjusted its activity to the level of portal blood sugar and was not inhibited by glucose-6-phosphate. The glucokinase of liver was greatly reduced in the diabetic animal and in starvation. Its reappearance after insulin could be hindered by inhibitors of protein synthesis, but ATP had a counter-inhibitory effect.

The next paper by *Leonard Madison* was on *The role of insulin in controlling carbohydrate metabolism in the liver.* He indicated that when counter-regulatory reactions to hypoglycemia were prevented, insulin could produce an immediate significant change in the release of glucose by liver in normal and diabetic animals, and thus exert some control over the liver in the regulation of blood sugar level during the ingestion of carbohydrate. Under the influence of endogenous insulin the liver disposed of a significant part of ingested glucose. He questioned the validity of isotope-dilution technics in defining quantitatively, the disposal of glucose by the liver under conditions of glucose loading.

P. A. Bastenie in discussing the second part of the symposium reported that in acute experiments on anaesthetized dogs, an inhibitory effect of insulin on liver glucose balance was not detected unless added to a glucose load, when it increased the inhibitory effect of hyperglycemia. An acute deficiency of insulin produced by neutralization with antibody, increased glucose output by liver and restoration of insulin restored the glucose output to low values. However, if the blood sugar was kept at normal levels, massive doses of insulin gave only a minor reduction in hepatic glucose release.

G. Weber indicated that adrenocortical hormones act as inducers of gluconeogenic enzymes whereas insulin blocks this enzyme synthesis. Glucose itself does not directly inhibit the enzyme synthesis but does this through insulin secretion. *G. Hetenyi* pointed out that both the net and tracer rates were required for the determination of hepatic glucose uptake and took issue with Madison's criticism of the isotope methods for determining glucose production or uptake.

L. Lambotte in perfused and intact dog livers found that insulin led to uptake of potassium by the liver within a few minutes regardless of whether hepatic uptake or release of glucose was occurring. In response to a question of *O. Wieland, Madison* stated that studies on isolated, perfused dog and rat liver showed that the action of insulin on glucose uptake was direct and not a consequence of decreasing the level of free fatty acids in blood. In reply to *R. Levine, Madison* indicated that when isotopic dilution technics are used alone, and not along with balance studies, hepatic glucose utilization cannot be differentiated from peripheral glucose utilization. He also pointed out the difficulties in interpreting the results of the isotope dilution technic when dealing with the hepatic cell.

The next paper on *Insulin and the metabolism of adipose tissue* was by *A. E. Renold, O. B. Crofford, N. Bürgi and E. R. Froesch.* They showed that insulin accelerates glucose transport across the cell membrane in adipose tissue and, with abundant hexokinase in the cell, glucose-6-phosphate is formed. This enhances all metabolic pathways for glucose-6-phosphate. Most of the physiologically meaningful effects of insulin on adipose tissue are secondary to this glucose-6-phosphate formation. Decreased lipolysis is one of these. The second half of the paper dealt with *in vitro* effects of insulin distinct from its effect on glucose uptake. In isolated adipose tissue preparations, release of free fatty acids and glycerol could be inhibited by insulin even when glucose was absent from the medium. Effects of insulin on glycerol release by adipose tissue from fasted-re-fed animals or by adipose tissue stimulated by lipolytic agents were observed in vitro, but it was not established that they occurred or were important in vivo.

In the next paper *A. Dorfman* dealt with *The metabolism of connective tissue ground substance.* Ground substance, in addition to providing some mechanical support, forms the chemical milieu in which transport of metabolites is carried out between cells and circulation. It varies in different tissues. The author reviewed the chemical nature of mucopolysaccharides, and dealt with the biosynthesis of the acid mucopolysaccharides, showing that glucose serves as a precursor of both the glucosamine and glucuronic acid moieties of hyaluronic acid. He indicated that the enzymatic reactions involved in the conversion of glucose to hyaluronic acid were reasonably clear but that there were still a number of questions concerning the mechanism of biosynthesis of the chondroitin sulphates. The metabolism of acid mucopolysaccharides was also discussed. Induction of alloxan diabetes in rats resulted in a decreased turnover of hyaluronic acid and chondroitin sulphate. The effects of other hormones on the biosynthesis of some of these compounds were also considered.

VASCULAR DISEASE

Symposium Number 3a involved a panel discussion opened by presentation of papers by the participants. The first paper, by *L. W. Kinsell* and *G. Schlier,* was concerned with *Dietary fat in diabetes mellitus with particular reference to vascular disease.* Evidence was presented which emphasized the importance of dietary control in modifying the hereditary tendency to atherosclerosis of the diabetic. Obesity should be eliminated or prevented. A high-calorie, high-carbohydrate diet tended to hyperglyceridemia. In their experience, favorable results were obtained with a low-calorie diet in which total fat supplied 40 to 50 per cent of the calories, not more than one fifth of which were provided by saturated fats and not less than two fifths by polyunsaturated fats, with adequate protein for growth and repair. They suggest a possible ideal plasma cholesterol of less than 100 mg. per 100 ml. for adults.

H. C. Knowles discussed *The control of diabetes and the development of vascular disease.* He analyzed the reports in the literature on the relation between the occurrence of vascular changes and the degree of diabetic control and pointed out the difficulties in assessing control. He believed that the information was as yet not sufficient to permit the conclusion that the incidence of these changes was related to the control of the condition. He could not recommend control to the point of reducing a patient's normal activities when the value of that control was not fully established.

The next paper by *Y. A. A. Larsson* divided diabetics into three groups instead of two according to the age of onset. He pointed out that the manifestation of vascular disease in diabetes is a mixture of specific, more or less specific and nonspecific vascular changes. The correlation between duration of diabetes and the occurrence of vascular changes appeared to be well established for the first fifteen years duration, but for the next fifteen years the relation between duration and angio-

pathy was not as evident. There was variation in the severity of the vascular changes within each type of diabetes. Diabetic micro-angiopathy often was present early in the course of clinical diabetes but there was a latent period between the onset of the condition and the appearance of signs of vascular lesions. He suggested that strict control might be given a more serious long-term trial.

Priscilla White considered that microangiopathy, the specific lesion of diabetes, was the most important cause of morbidity and mortality in the young. She pointed out the genetic origin of the vascular lesions and indicated that according to Warren and LeCompte 25 per cent had atherosclerosis when the duration was one and one-half years, 70 per cent when the duration was five years and 85 per cent when it was fourteen years. Clinical evidence of vascular lesions was rarely found under the age of twenty or under a duration of fifteen years. She indicated too, that with the present insulins, the restoration of normal values for blood glucose at all times without the production of hypoglycemia is nearly impossible and that it is hard to evaluate the degree of control in children and teenagers.

RETINOPATHY AND PITUITARY FUNCTION

Symposium Number 3b, the second panel discussion, dealt with *Approaches to treatment of retinopathy by modifying pituitary function.*

J. L. Born discussed the effects of *Pituitary irradiation with heavy particles in diabetic retinopathy.* Of the patients with progressive diabetic retinopathy who received such irradiation about 55 per cent of those evaluated showed stabilization of the retinopathy for periods ranging from four months to six and one-half years. The stabilization seemed to be better correlated with reduced insulin requirement than with the degree of hypopituitarism. The patients whose retinopathy was stabilized were distributed through the dose ranges from 8,000 to 33,000 rads.

R. A. Field dealt with the *Approaches to treatment of retinopathy by modifying pituitary function* by hypophyseal stalk section and by heavy particle irradiation. There was an amelioration of rapidly progressive hemorrhagic diabetic retinopathy in 67 per cent of his selected cases but the complications of diabetes outside the eye appeared to remain unchanged. With heavy particle irradiation of 12,000 to 15,000 rads at one sitting 73 per cent of patients gave evidence of hypopituitarism. Of these, roughly half showed stabilized or improved vision. The conclusion was that there was a positive correlation between the beneficial effects of irradiation and the development of hypopituitarism.

R. Fraser, D. W. Hill, G. F. Joplin, F. Doyle and N. Oakley dealt with *Pituitary ablation by needle implantation of Yttrium-90 for diabetic retinopathy.* Patients receiving needle implants of Y-90 were studied for five years. Controls showed increasing impairment of visual acuity whereas the treated group showed improvement. There was little improvement in either group as far as exudates and retinitis proliferans were concerned but there was improvement, in the implanted group, with respect to hemorrhages, the formation of new vessels and venous dilatation. There was a troublesome incidence of rhinorrhea in the needle-implanted patients. The results suggest that the progress of diabetic retinopathy may be arrested as well by partial suppression of pituitary function as by its total suppression.

R. Luft discussed the indications that HGH (human growth hormone) plays a *Role in diabetes mellitus and diabetic retinopathy.* He pointed out that it is not known which pituitary hormone or hormones must be removed in order to change the natural history of diabetes but that growth hormone has received much attention. HGH is diabetogenic in human subjects but endogenous insulin protects against its major effects. He presented evidence that some pituitary factor and insulin are both important in protection against the diabetogenic effects of HGH. In healthy subjects HGH increased blood insulin levels, though at the same time it decreased glucose tolerance. In acromegalic patients with normal glucose tolerance but presumably high levels of HGH, the infusion of glucose gave a very large increase in plasma insulin levels.

In the discussion, *Born* indicated that in the range of dosage used there were no serious problems but that with higher doses there was some incidence of extraocular palsy. *L. E. M. Volk* stated that with the anabolic steroid decadurabolin, improvement comparable to that reported for pituitary destruction could be obtained. *L. W. Kinsell* brought out the fact that the better diabetic management and follow-up itself could result in favorable changes. *Fraser* concurred in this. He stressed the importance of randomly chosen controls to show the effect of androgens or other treatment and emphasized the difficulties in running a control series. At least a one year follow-up was used in their series. *Field* indicated that accompanying kidney disease continued and that there was a tendency for thyroid function to improve with time after the pituitary damage, the protein-bound iodine rising from 0.5 micrograms per 100 ml. to two or three micrograms per 100 ml. in six months to a year. *I. Graef* called attention to the effectiveness of adrenalectomy, which also reduced insulin requirements. *Luft* pointed out that the major hazard following hypophysectomy is hypoglycemia but that reduction in plasma proteins as a consequence of reduced albumin synthesis may be associated with edema in young female diabetics. Anabolic steroids restored the albumin values and caused the edema to disappear. *P. Miller* called attention to the difference in prognosis with different types of retinopathy and stressed the importance of matched controls.

INSULIN IN BLOOD

Symposium Number 4 was on *Insulin in blood. F. C. Goetz* discussed *The validity of different methods for the assay of insulin in blood.* He indicated that any insulin assay procedure must be sensitive, specific, precise, and any interfering factors must be known. The present state of most insulin assays does not allow conclusions regarding these matters. There is some doubt that the immunoassay procedures measure all insulin present and in immunoassay methods it is possible too, that the antibody might not recognize all forms of endogenous insulin in plasma. The bioassay procedures, as usually used, have 95 per cent confidence limits from half to approximately twice the estimated potency of the sample. The specificity of the fat pad assay is questionable since insulin-like activity persists after pancreatectomy in dogs. In general the values obtained with the fat pad are higher than those obtained with the diaphragm assay. He reported that with acid-alcohol extraction there was an increase in assayable activity by immunoassay in plasma

samples obtained after glucose.

The next paper by *H. N. Antoniades* was on the *Extrapancreatic regulation of insulin activity in human beings.* He reported that the insulin in blood circulates in free and bound forms, the free insulin, which is biologically active, being released by the pancreas and the bound insulin, which is biologically inactive, being generated (i.e. combined) at extrapancreatic sites. He postulated that the utilization of this insulin and the formation of bound from free may be catalyzed by tissue factors. Transformations of insulin thus could occur at the tissue level and would assist in regulating insulin activity. Anything affecting the rate of activation of insulin or its utilization or inactivation could influence insulin effects. The suggestion was made that the hyperglycemia in some types of diabetes may result from abnormalities in this insulin-regulating mechanism.

In the discussion, *J. Bornstein* listed a series of objections to the immunoassay procedures. He also indicated, in connection with the bioassay procedures, that several components of plasma have been isolated which lack metabolic function yet enhance the effect of insulin on the uptake, utilization or oxidation of glucose and so affect the results of the assay. *P. Sonksen* mentioned that adsorption on glass, even in buffer containing gelatin, gave significant loss of insulin which did not occur when albumin was used. The statements of Bornstein concerning the immunoassay procedure were challenged by *F. Good* and by *J. L. Rizzo.* The latter noted that iodination to the extent of producing biologically inactive insulin did not affect the binding capacity of guinea pig insulin antibody. *E. R. Arquilla* added that iodine and other halogens interfere with the combination of antibody to homologous haptens. Most of the iodine is attached to aminoacids 14 to 19 of the A chain of insulin. These therefore would not appear to be implicated in the immunoassays and may account for low results. *G. M. Grodsky* found no increase in antibody-neutralizable insulin after extraction and *N. Samaan* emphasized the lack of relation of "atypical" insulin to Antoniades "bound" insulin. "Atypical" insulin does not disappear after oral glucose and "atypical" insulin changes little after intravenous tolbutamide.

The paper by *J. Steinke* and *J. S. Soeldner* was on *Serum insulin-like activity in health and disease.* They found that values obtained with the Morgan and Lazarow immunoassay procedure, were consistently lower than values obtained by the fat pad assay using C-14-labeled glucose, and that there was no direct relationship between the values obtained by the two methods. Only about one third of patients with islet cell tumors showed elevated serum insulin levels at the time of hypoglycemia. Following tolbutamide, the serum insulin-like activity rose five to ten times. In maturity-onset diabetics, normal or elevated serum insulin-like activity was found by all assay methods. In the early stages of growth-onset diabetes there was persistence of insulin-like activity as indicated by the rat adipose tissue assay but uniformly low levels were obtained using the rat hemidiaphragm procedure. The pancreatic insulin levels were higher in fetuses from diabetic patients exhibiting high serum ILA, the activity remained essentially unchanged after acid ethanol extraction but in those exhibiting low ILA, the insulin activity increased markedly after acid ethanol extraction. In both types large quantities of antibody were found. They concluded that antibodies to insulin are not necessarily the cause of insulin-resistance though associated with it.

J. K. Davidson described prediabetics or early diabetics with insulin levels in excess of 1 milliunit per ml. of native (untreated) serum in the fasting state, the levels remaining high three hours after a glucose load. Normal subjects had native serum insulin levels less than 0.6 milliunit per ml. in the fasting state which rose to a peak at one hour and then declined. The total insulin activity of serum after acid-alcoholic treatment and dialysis, was not higher than normal in the serum of these prediabetics and early diabetics which suggested that it was the fraction of the total insulin activity that was evident in native (untreated) fasting serum which may have been elevated. In insulin-resistant diabetics the insulin activity was low in native serum and very high after acid alcoholic treatment and dialysis.

The next paper by *E. Samols* was on *The immuno-chemical aspects of insulin.* Insulin I-131 rapidly disappeared from the blood in normal subjects and was not bound to any particular protein but in subjects who had received insulin for a few weeks it persisted much longer and was bound to certain serum proteins.

He discussed the immunoassay of insulin, and indicated that the antibody-neutralizable insulin behaved according to the classical concepts of glucose-insulin homeostasis, though often the plasma insulin changes lagged behind those of glucose. A wide range of plasma insulin was found in young adults and a reduction in levels was observed after a low carbohydrate diet for three to five days. Obese subjects were found to have higher insulin levels and lower glucose levels than nonobese. Evidence was presented that a rise in endogenous insulin influenced the rate of disappearance of blood glucose. Different types of insulin response to intravenous tolbutamide were noted in a group of mild diabetics. A brisk, early, excessive insulin response may precede a later delayed response and finally an exaggerated endogenous insulin response. It was suggested that sequential changes in function of the islets in diabetics might be recognized by insulin assay.

Following this paper, discussion was opened by *P. Moloney,* one of the pioneers in the immunochemistry of insulin. He intimated that guinea pig anti-ox insulin antibody can be precipitated by rabbit anti-guinea pig globulin and this precipitate will specifically absorb insulin. If such an absorbing immuno-precipitate can be prepared having a zero insulin blank, then this would be of use in investigations on insulin and insulin-like substances in blood. *L. E. Miles* reported that in obese patients the plasma insulin response was exaggerated. He did not find any increase in the measured immuno-chemical insulin after acid alcoholic extraction.

ANGIOPATHY AND ITS CLINICAL CORRELATIONS

Symposium Number 5 was on *Angiopathy, clinical correlations. The pathology and pathogenesis of the disseminated angiopathy of diabetes mellitus* was presented by *H. G. Blumenthal, S. Goldenberg and A. W. Berns.* They proposed the concept that auto-immune phenomena, involving as antigen a changed endogenous insulin, are present in diabetes and both the angiopathy and the metabolic changes in maturity-onset diabetes might be accounted for by the subsequent reac-

tions. When rabbits were immunized with an insulin-adjuvant mixture and challenged with small amounts of insulin there resulted proliferative vascular lesions, nodular glomerulosclerosis, hyaline islets and retinal microaneurysms. While this appeared to support the idea of an association of angiopathies with immune reactions, the animals immunized against insulin did not develop diabetes.

In the discussion *Blumenthal* said that the mutations involving beta cells might occur as an aging phenomenon. He indicated too that a second immune response to exogenous insulin might intensify any effect of this.

The next paper by *K. Lundbaek* dealt with *Nephropathy in diabetic subjects.* In 35 per cent of cases of diabetes diagnosed before forty years of age renal insufficiency was an important cause of death, whereas in those diagnosed later it was important in only 12 per cent. Electron microscopic studies showed that the histopathogenesis of diffuse glomerulosclerosis differed from that of the nodular type. Electron microscopic pictures of serial biopsies of kidneys from recent growth-onset diabetics were not clearly different from those of normal subjects, nor was there any great difference before and after control of the metabolic state in diabetic patients. Changes ascribed earlier to chronic infection may actually have resulted from the vascular anomalies. Both glomerular and tubular functions were reduced as the nephropathy progressed.

J. Syllaba reported that ultracentrifugation of serum showed atypical components of macromolecular globulins in 82 per cent of diabetics with clinical nephropathy. The concentration of component G corresponded with the clinical severity while that of the atypical component did not. The pathological sedimentation pattern of atypical serum macroglobulin appears to be reversible on treatment with anabolic steroids.

The next paper by *S. I. Caird* was entitled *Prognosis for vision in diabetic retinopathy.* Studies at the Radcliffe Infirmary and in Boston led to the conclusion that in patients with retinopathy and initially good vision in both eyes the deterioration in vision develops at about 5 per cent per annum if the retinopathy is of mild or moderate degree, 11 per cent per annum in proliferative retinopathy in growth-onset diabetics, and 25 to 30 per cent per annum in maturity-onset diabetics. For gross visual deterioration the rates are 2.5 per cent, 7-9 per cent and 10-15 per cent per annum.

The paper by *J. H. Lawrence, J. L. Born, J. A. Linfoot* and *C. T. Tobias* dealt with *Hypophyseal heavy particles in diabetic retinopathy.* After pituitary irradiation with heavy particles there was stabilization of the retinopathy in about 55 per cent. This did not seem to be dependent on complete suppression of pituitary function but rather on the drop in insulin requirements. With the dose range of 8,000 to 15,000 rads given in twelve days there were no complications. The patients with *retinitis proliferans did not respond well.*

Caird noted that over a five-year period there was improvement without special treatment in 36 per cent of eighty-two eyes with microaneurysms alone and in 27 per cent of 209 eyes with hemorrhages and/or exudate. Both *Lundbaek* and *Lawrence* stressed that pituitary irradiation must be considered an experimental procedure at present.

A. I. Winegrad dealt with *Alterations in aortic metabolism and diabetes.* Rabbit thoracic aorta was incubated with uniformly labeled glucose C-14 in bicarbonate buffer under aerobic conditions. Lactate production accounted for a large part of the glucose uptake. Insulin did not increase the lactate production or C-14-O_2 production but very small increases in glycogen were noted. In the aortic intima and media from diabetic rabbits there was a reduction in glucose uptake and lactate production. The glucose utilization was brought back to normal only forty-two hours after insulin injection into the diabetic rabbit. The suggestion was that impaired phosphorylation of glucose was responsible for the change and that because of the inability to use glucose, the precursors necessary for phospholipid synthesis would not be available. The results supported the idea that insulin deficiency may play a part in the vascular lesions in diabetes.

In the paper by *S. E. Rees,* the *Pathogenesis of pathognomonic and non-specific microvascular lesions in diabetes,* was discussed. He studied gross and fine structure of small blood vessels from patients in various stages of diabetes. In juveniles, there were dilatation of capillaries and post-capillary venules with increased tortuosity and diminished tone, mural cell or pericyte degeneration predisposing to microaneurysm formation where the endothelial cells had proliferated, and arteriolar-venular shunt formation. The more distal capillary beds became ischemic and new vessels formed in response to this. Using serial renal biopsies in young diabetics the progression of diabetic glomerulosclerosis was followed. Capillary dilatation and tortuosity were evident and in efferent arterioles the basement membrane became thickened. Nodules formed from excess basement substance in the mesangium. This process was accelerated in poorly controlled diabetics and possibly by the over-production of basement membrane substance by paths independent of insulin.

In answer to a question by *K. Jahnke, Rees* described fibrillar degenerative change in the elastic tissue of the capillaries of the ear lobe before thickening of the basement membrane in prediabetics. This was found also in arterioles similar to the afferent arterioles of glomeruli. Early nonspecific evidence of arteriolar sclerosis was the first sign of the prediabetic state. *Winegrad* reported that L-xylulose measurements in serum were consonant with the view that glucose utilization by way of the uronic acid pathway was unimpaired or increased in diabetes mellitus. *J. Ireland* suggested taking many pictures of serial cuts through the mesangium to decide when the center of the mesangium of the axial region had been cut. This would establish that a true cross section of the capillary wall had been made and would prevent inaccurate results.

Continued in the June issue