The Role of Insulin in the Pattern of Diabetes Mellitus

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Historical Background

Diabetes mellitus has been known to exist from time immemorial. It was recognised in ancient Egypt and has been found described in Indian Sanskrit Verdic literature as "the passing of urine with honey". Aretaus (1st century A.D.) gave the first classical description of the disease and laid emphasis on the large amounts of urine passed — hence the term diabetes, from the Greek for siphon. It was left to Thomas Willis who, recognizing the importance of sugar in the urine, added the term mellitus — Latin for honeyed — in 1674, thereby distinguishing it from the insipid variety. Chevreul in 1815 finally defined the nature of the reducing substance in urine of diabetics to be glucose.

Later, Cawley Bright, and others pointed out the association between Diabetes mellitus and changes or malfunctioning of the pancreas. This was confirmed in 1889 by the German physiologist Oscar Minkowski; he and Von Mering found that pancreatectomy in dogs caused a condition which strikingly resembled Diabetes mellitus in man. In 1869 Paul Langerhans had previously discovered special specific cells which appear as nests or islets in the pancreas, and in 1916 Sir Edward Shafer suggested that these islets secreted some substance controlling the metabolism of carbohydrates, and coined the name INSULIN for this substance. For many years, attempts to extract and isolate insulin met with nothing but failure and frustration. Eventually, a young orthopaedic surgeon, the late Frederick Banting, working with Charles Bets, then a medical student, in Macleod's laboratory in Toronto, succeeded in 1921 by ligating the pancreatic duct thus producing degeneration of the acinar part. The pancreatic beta cells had at last been made to yield their valuable hoard. Insulin was first used on a human subject on January 11, 1922, the patient being a young diabetic doctor, Joe Gilchrist. These extracts were still unpredictable in terms of toxicity and potency and it was only in 1926 that insulin was first obtained in its crystalline form by Abel and Geiling. In 1955 Frederick Sanger succeeded in elucidating the chemical structure for which he was awarded the Nobel Prize 3 years later. Claus Hocman in Pittsburg in 1963 succeeded in synthsizing insulin in the laboratory for the first time.

Structure, Action and Role of Insulin in the Metabolic Pathways.

The insuling molecule consists of two polypeptide chains — a glycine chain, Fraction A consisting of 21 amino-acids and in internal S-S bridge; and a phenylalanine chain, Fraction B containing 30 amino-acids; the chains being connected by disulphide (S-S) bridges of cystine residues. Up to recently, the rate of insulin releases appeared to be mainly a function of the blood glucose concentration; it falls with hypoglycaemia and rises with hyperglycaemia. It is not known whether the insulin concentration of the blood perfusing the pancreas also serves as a negative feedback mechanism. The beta-cell membrane is apparently freely permeable to glucose but it is uncertain whether the release of insulin is controlled directly by the intracellular concentration of glucose or of one of its metabolites. Lately it has been proved that plasma insulin levels are higher with intestinal-intrajejunal - administration of glucose than with intravenous glucose, despite much lower blood glucose levels. This suggests that factors other than arterial glucose con-

centration may be involved in the regulation of plasma insulin levels. The most likely explanation seems to be the release of a humoral substance from the jejunal wall during glucose absorption which acts, along with a rise in blood glucose, by stimulating the release of insulin from the beta-cells. Insulin is stored in the beta-cells as encapsulated granules (Hartroft & Wrenshall, 1955).

The E/M has shown that liberation of the granules from the cells is a relatively simple process. The earliest change is a margination along the plasma membrane of the cell by the granules encased in their smooth membranous sacs. The smooth sacs encascasing the granules fuse with the cell membrane, and rupture; the granules are liberated into either the intercellular or pericapillary space. The granules apparently undergo rapid dissolution since they are no longer visible by E/M in the extracellular space. This process of simple ejection of the granules into the E.C.F. is called emiocytosis. The synthesis of the beta granules appears to occur within the ergastoplasm of the cell.

The main effect of insulin is of course the reduction in the blood glucose level mainly by encouraging peripheral utilisation of glucose especially by the muscles and by favouring glycogenesis in muscle and adipose tissue. In contrast with the well established action of insulin on muscle and adipose tissue, its effects on glucose metabolism in the liver remain inconclusive and a matter of controversy. A large body of evidence indicates that insulin plays a role in controlling the hepatic output of glucose; the question whether insulin stimulates hepatic glucose uptake remains largely unsettled. The magnitude of the effects and sites of action of insulin on hepatic glucose metabolism are still uncertain. DeBodo has recently determined that insulin inhibits glucose production by the liver. Formerly it was thought that insulin achieved the promotion of glucose utilisation by enhancing the hexokinase reaction (Cori 1946) wherein glucose is converted to glucose-6-phosphate, the only form in which it can be utilised for energy production, or stored as intracellular glycogen. It appears now, that there is no ground for supposing that insulin activates hexokinase or removes a physiological inhibition of this enzyme. Levine and Goldstein in 1960 have postulated that insulin primarily stimulates the transfer of glucose across the cell membrane rather than acting on specific intracellular enzymes. Muscle cells, fibroblasts and adipose tissue do not permit the rapid free entry of sugars; rather the cell membrane possesses a specific transport system which carries glucose into the cell interior at a rate greater than can be explained by simple diffusion. This transport system requires the presence of insulin; insulin in effect can be imagined to open a sort of trapdoor which permits glucose to enter freely. Neurones and erythrocytes can transport glucose across their cell membrane without insulin Cells of the intestine, liver cells and perhaps also those of the renal tubules can transport glucose without insulin. If there is any direct action of insulin on carbohydrate metabolism of the liver cell, it does not seem to be related to cell membrane transport (Levine 1965).

It must be remembered that insulin is not the only agent which facilitates sugar transfer. Clinical experience and the more recent experimental work by Ingle have shown that muscular exercise can also bring about this transfer in the absence of insulin.

If glucose does not easily enter the cell there is a decrease in the normal intracellular metabolism of glucose which in turn leads to disturbance of liquid and protein metabolism. Chain (1960) has also suggested that insulin exerts a specific stimulating effect on a number of energy-requiring reactions involved in the synthesis of glycogen, fat and protein. Once intracellular glucose has become phosphorylated to glucose-6-phosphate it cannot leave the cell. Glucose-6-phosphate may either be converted to glycogen or be broken down via the classical Embden-Meyerhof pathway to form acetyl-CoA which may either provide energy via

the Krebs cycle or act as substrate for the syntheses of fat and protein. In adipose tissue, and to a lesser extent in the liver, there is an important alternative metabolic pathway for glucose-6-phosphate, the hexose monophosphate shunt. Insulin may also have a direct effect on protein metabolism by stimulating directly protein synthesis. Insulin is inactivated by degradation, this is in contrast to the inactivation of the steroid hormones in which the conjugation mechanism plays an important part. The degradation of insulin appears to be due to a preliminary reductive cleavage of the S-S bond by a glutathione-insulin-transhydrogenase and a subsequent hydrolysis of the resultant A & B chains. Hypophysectomy decreases this degradation and this may in part account for the insulin sensitivity in panhypopituitarism.

The Use of Insulin in the various patterns of Diabetes mellitus.

Diabetes mellitus is a syndrome with a variety of causes, all having in common hyperglycaemia from an impaired glucose catabolism, with consequent glycosuria, polyuria and dehydration. Secondary alterations in fat and protein metabolism lead to tissue wasting, ketosis and coma in the uncompensated state. The central metabolic lesion lies in the underutilisation of glucose as a consequence of an absolute or, more often, relative lack of insulin. Following Banting and Best's brilliant contribution, it soon began to be realised that insulin is not the whole solution to the problem of Diabetes mellitus. Diabetes research has shared in the general ferment of biochemical investigations during the past one or two decades, yet its aetiology remains as much as an enigma today as forty years ago; indeed even more complexities are nowadays evident. Diabetes mellitus not only manifests itself in the form of metabolic abnormalities but is also expresed as a strong susceptibility to arteriosclerosis and to certain rather distinctive lesions in the kidneys, retinas and elsewhere which are characterized chiefly by microangiopathy. There is a definite genetic disposition to the disorder but a syndrome with similarities at least in the metabolic aspects makes its appearance following pancreatectomy or chronic pancreatitis and in conditions associated with excessive secretion of certain hormones notably growth hormone and the glucocorticoids. The exact mechanism responsible for insulin insufficiency in Diabetes mellitus is unknown. It has become increasingly evident that a primary defect in beta-cell function cannot be responsible for the insulin insufficiency in the majority of patients. Theoretical possibilities include blockage in the formation of insulin or in its release from the pancreas, blockage of its passage through any one of the various membranes, neutralization in the bloodstream, excessive degradation, excessive amounts of opposing hormones and inability of the tissues to accept or to utilize it. The diminished reserves of insulin in the islets of many diabetic patients appears to develop secondarily to a chronic drain induced by extrapancreatic factors such as inactivators, inhibitors and perhaps antagonists of insulin. The possibility that Diabetes mellitus may be due to the inheritance of abnormal plasma proteins — insulin antagonists has been raised by Bornstein and J. Vallance — Owen (1964). However, there is still lack of definitive evidence and it is becoming increasingly difficult to envisage Diabetes mellitus as a disease due to such a single factor. F.G. Young has brought out evidence supporting the view that an abnormally high level of serum Growth hormone may be an important factor in some cases of diabetes. This has been confirmed by Randle.

Diabetes mellitus exists chiefly as two main clinical varieties: Type I is the diabetes of acute onset in young usually thin people rapidly leading to severe ketonaemia, coma and death unless treated with insulin. This is known as the juvenile type of diabetes. Assay of the insulin activity of the plasma, using the rat diaphragm or the rat epididymal fat pad, shows none to be present, this was first carried out by

Bornstein & Lawrence, then by Vallence Owen. Type II is the diabetes of gradual onset, in older, usually obese people and occurring more frequently in women. This is due to a relative rather than an absolute insulin lack. Plasma assay shows near normal insulin activity. This is the maturity onset type of Diabetes mellitus.

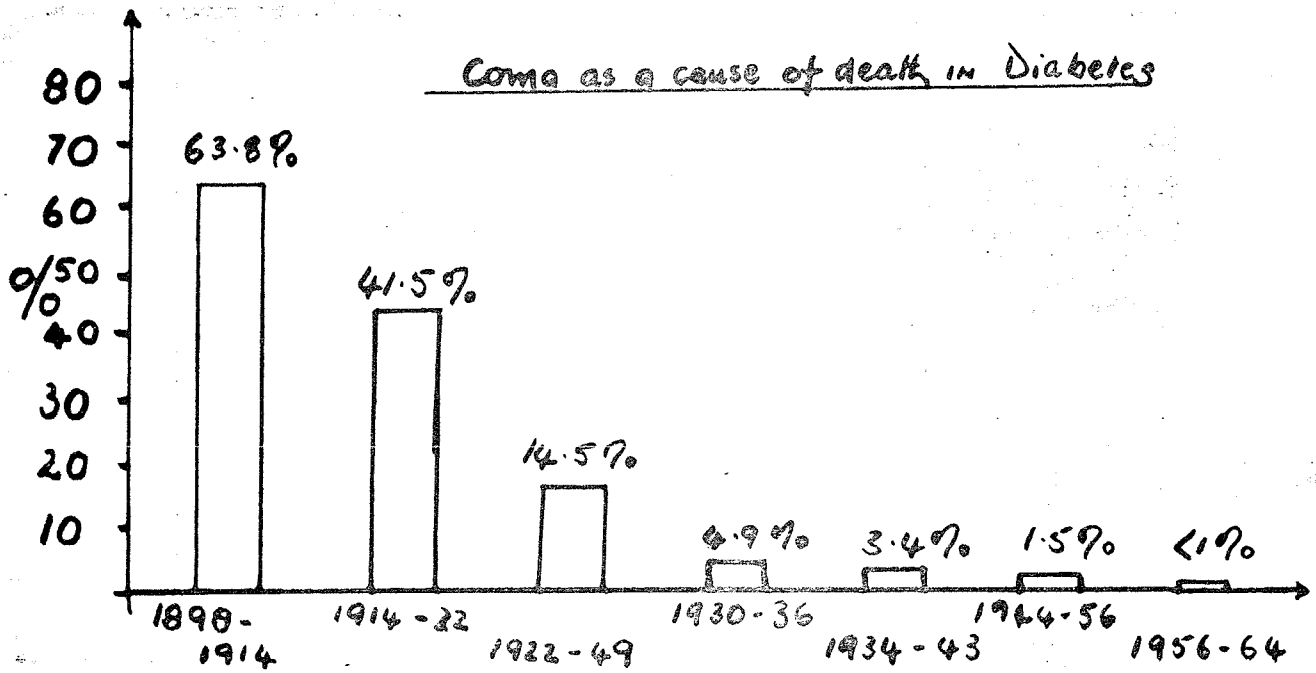
Juvenile diabetics require treatment by both insulin and diet, and need to have these matched to the amount of physical exercise they take daily. Clearly a heavy manual worker will need more insulin and more calories than a clerk. For adequate control a regular amount of activity and a regular diet are essential, and certain occupations, such as commercial travelling, are best avoided. Each case must be judged on its own merits and adequate treatment administered per individual: as the juvenile type of diabetes is particularly unstable. Besides physical activity of the diabetic, when considering the number of calories needed, such factors as age, sex, weight and height must also be taken into consideration. Carbohydrates must not be restricted too rigorously, as too little promotes gluconeogenesis, imparts glucose tolerance and decreases insulin sensitivity. Dunlop recommends 100 grams of carbohydrates for every 1000 calories of the diet. Perhaps nowhere better than in Dr. Lawrence's article "I have lived for 40 years the life of a diabetic patient" can one understand and appreciate the the great role which insulin has come to play in the day to day life of a juvenile diabetic. Before 1921, the juvenile diabetic used to eke out a miserable existence for a few years on semi-starvation dietary regime. Dr. Lawrence who was up till recently Director of the Diabetic Clinic in King's College Hospital, London, describes how he had to give up a promising surgical career and go to Florence, where he was always on the brink of passing into coma and unable to do any solid work. The discovery of insulin changed completely the whole aspect of his life and enabled him to further his medical career as well as to participate most fully in the world around him.

The maturity onset type of diabetes is usually well controlled with diet, with or without the administration of oral hypoglycaemics; sulphonylureas or the diguanides. Both phenformin and metformin — the diguanides — and carbutamide, tolbutamide and chlorpropamide — the sulphonylureas, are not without toxic side effects. Diabetics treated on such a regime are those having a stable type of diabetes who are not prone to develop ketosis. Patients with adult-onset diabetes who cannot be controlled satisfactorily by diet alone or with an oral hypoglycaemic agent, also require insulin. Patients with stable diabetes controlled by diet or an oral hypoglycaemic agent may become uncontrolled and sometimes ketoacidotic if they develop an acute or chronic infection, suffer physical injury, undergo an operation under anaesthesia or become emotionally upset. Under such circumstances they may require insulin temporarily.

A number of insulin preparations is now available differing in time of onset, peak of activity and duration of effect. The first to be produced was Soluble Insulin which has a powerful, rapid but relatively fleeting hypoglycaemic effect, in fact it starts to work in ½ hour, reaches its peak in 2-3 hours and the effect is over in 6-8 hours. It imitates roughly the increased secretion of insulin by the normal pancreas after meals. Longer acting insulins were later introduced by combining insulin with protein and buffer; such preparations include isophane insulin (N.P.H.) globin insulin, Protamine Zinc insulin; the latter was introduced by Hagedorn in 1936. Their effect on the blood sugar is delayed, relatively smooth and prolonged. P.Z.I. taken 4-6 hours to start acting, reaches its maximum in 15-18 hours and the effect is over in 24-32 hours. Globin insulin and N.P.N. are intermediate in action between Soluble insulin and P.Z.I., their effect starts within 2-4 hours and is over in about 24 hours. In 1952 the insulin zinc suspensions (I.Z.S.) were introduced in Copenhagen by Hallas-Moller. It is prepared in either the quick-acting amorphous form; semilente, having much

the same time of action as the globin variety, or the slow acting crystalline form; ultralente, very similar in effect to P.Z.I., but acts a little more rapidly and probably for a shorter time. A third variety is I.Z.S. lente, a cloudy preparation containing 3 parts of I.Z.S. semilente to 7 parts of I.Z.S. ultralente; it starts to act within 1 hour and lasts for about 24 hours. The main advantage of I.Z.S. is that it contains no foreign protein and so is less likely to cause local or general sensitization phenomena. Dunlop recommends that patients who do not require more than 40 units of Plain insulin daily can be well controlled with one subcutaneous injection of I.Z.S. lente before breakfast a day. The vast majority of diabetics requiring insulin are, according to Dunlop, best controlled by P.Z.I. and soluble insulin given before breakfast, about $\frac{1}{3}$ of them will require an additional injection of Soluble insulin before the evening meal. Recently, two new preparations have been put on the market. Actrapid novo insulin, which acts within 15-30 mins. and lasts for about 6 hours; and Rapitard which contains no foreign retarding agents, starts within 15-30 mins. and lasts for about 18 hours. The doses required, vary from patient to patient, and each requires individual management. The education of the diabetic is essential to success in treatment, if possible he must be taught to correlate diet, exercise and insulin doses with various conditions of life.

Diabetic coma is always a medical emergency, the patient should be hospitalized without delay and as soon as he arrives the patient should receive 50 units of soluble insulin intravenously and a further 50 units subcutaneously. Supportive therapy is of course instituted at the same time, this includes amongst other things intravenous infusion with isotonic saline, antibiotics and replacement of Potassium if necessary. After blood glucose estimations, the patient is to be treated accordingly with further doses of soluble insulin until stabilized. Further appropriate treatment with other insulin preparations is of course later instituted. Diabetic coma as a cause of death in diabetics, has nowadays fallen down rapidly from about 64% in the early 1900's to below 1%; the advent of insulin, together with increased advance in the field of biochemistry has profoundly altered its incidence.



Up to 50 years ago, most diabetic women were infertile; with the advent of insulin this too has changed to some extent and an increasing number are becoming pregnant. Insulin has rendered pregnancy relatively safe for the diabetic mother, in fact in pre-insulin days mortality was about 45%, now it is less than 1.5%; but the child is often born macerated and dead or dies soon after.

Insulin requirements usually increase sharply for the first trimester, remain steady for the second and usually increase for the third. It is particularly important to remember that the insulin requirements fall dramatically after delivery, so that the dose should be drastically curtailed as otherwise hypoglycaemia sets in. Infections also result in increased insulin requirements. Diabetes in children is exceptionally difficult to treat and keep under adequate control. Perhaps the most suitable regimen, according to Dunlop, is the daily injection of P.Z.I. and soluble insulin before breakfast and the administration of a second dose of soluble insulin before the evening meal. The dose of the morning soluble insulin is based on the pre-lunch and pre-supper urine tests, that of the evening on the pre-bed test and the dose of the P.Z.I. on the true-fasting urine test.

The Changing Pattern of Diabetes Mellitus

The pattern of the causes of death among diabetics has altered radically since the introduction of insulin in the early 1920's and that of anti-biotics in the early 1940's. The two major hazards of diabetes, ketosis and intercurrent infection can now be effectively controlled. Tuberculosis, which undoubtedly was responsible for the death of about half the patients 50 years or even a generation ago in many large city hospitals of the North, has now dropped to 0.2%. Diabetic coma has fallen from 64% to below 1%.

The prevention and treatment of the vascular complications is now the chief problem. Vascular disease in the heart, brain and kidneys is now responsible for at least 75% of the deaths in diabetics. In America the vascular component of diabetes occupies the sixth or seventh place as the cause of death and its ocular manifestations take more people to ophthalmologists than does any other disease and is the third leading cause of blindness in the U.S.A. The basic lesion in these vascular complications consists of a proliferation of the intimal endothelium and a thickening of the basement membrane which histochemical techniques have revealed to contain a high polysaccharide content. Possibly microangiopathy is due to a seepage of polysaccharides from the circulation through foci of capillary damage. This diffuse capillary disease may effect the kidneys resulting in nephropathy, one form of which is the Kimmelstiel-Wilson lesion, first described in 1963 and characterized by cosinophilic nodules in the glomerular tuft; the eyes-retinopathy, the most characteristic abnormality being the microaneurysm, first described by Mackenzie & Nettleship in 1877; and the nervous system — neuropathy. A long standing question still remains without a satisfactory answer. Are the vascular lesions a consequence of the metabolic derangement or are these independent defects? Mirsky, Ellenberg, Spiro and others are now of the opinion that the renal lesions in diabetes mellitus, as well as extra-renal micro-angiopathy are genetically determined features which are independent of rather than a a consequence of, the defect in carbohydrate metabolism. The hyaline changes in or beneath the basement membrane of the islet capillaries are regarded as an expression of the generalised microangiopathy and are responsible for the ultimate abnormalities in insulin secretion. S. Berson in the Banting Memorial lecture of the American Diabetic Association for 1964 states that the relationship between adequacy of control of the blood sugar level and the development of such pathological lesions remains difficult to evaluate because of the arbitrariness and subjectivity involved in deciding whether "good control" has existed. Although the control of Diabetes mellitus cannot be related

definitely to the development of vascular damage, a rapid and severe course of events may develop following a period of poor management. Clinical evidence seems to point out that inadequately treated patients are apt to develop these vascular complications earlier and to a severer degree. The hypophysis is also coming more and more into the picture since the case described by Poulsen in 1953, where a woman with severe diabetic retinopathy improved dramatically after post-parum hypopituitarism. Both Prof. Russell Fraser of the London Postgraduate Medical School and Prof. Luft of Stockholm, among others, have reported success in carefully selected cases of diabetic retinopathy by pitultary ablation; visual deterioration is usually halted and there is often a lessening of the active features. The incidence of peripheral vascular disease — atherosclerosis—is also higher and comes on at an earlier age in diabetics. The control of Diabetics. The control of Diabetes mellitus in patients with atheroscleresis is quite important especially in the presence of infection or of ulcerative or gangrenous lesions.

From the surveys carried out, it is calculated that there are about 50 million diabetics throughout the world. The first major published survey was carried out in the U.S.A.; at Oxford, Mass., by Wilberson & Krall (1947). Further surveys carried out include the Birmingham survey by ten G.P.'s in 1962; the Bedford survey in 1963 by Prof. Butterfield and lately one in Malta by Prof. J.V. Zammit Maempel. Wilkerson and Krall found a total incidence of 1.4%; Prof. Butterfield an incidence of 12% and Prof. Zammit Maempel in the pilot survey in Malta found the incidence of glycosuria to be 8.9% and the total incidence 19.9%. Insulin in no way affects the incidence of new cases, and as it staves off mortality, it increases the total population of diabetics. As there appears to be a hereditary factor in the development Diabetes mellitus, the numbers vulnerable from generation to generation increase. As death rates from general mortality fall and the number of elderly people rises, the incidence of Diabetes mellitus will also rise since it becomes more common with age. Prof. Butterfield in his report on the Bedford Survey writes that the medical services must be prepared to cater for the appearance of very large numbers of diabetics in the immediate future.

The pattern of the mortality has altered greatly since the advent of insulin. Mortality in the younger age groups has been substantially reduced since insulin began to be more available and be more widely used. For ages up to 35 years death rates are between 1/10 — 1/5 of the levels of the early 1920's The change in mortality from diabetes although due in major part to the introduction and increased availability of insulin, must also be attributed to the availability of anti-biotics and to the more rational approach of dietary treatment. The period of most rapid decline in mortality is the decade 1940-1950; this coincides with the introduction of the sulphonamides (1936 onwards), then of penicillin (mid. 1940's) and eventually of the broad spectrum anti-biotics. These provided effective therapy against infection to which the diabetic is so vulnerable and which upsets so readily his metabolic balance.

Over the past 40 years, Diabetes mellitus has changed from a progressive or even rapidly fatal disease into a controlled chronic disorder with mortality mainly confined to old age. This new picture dates from the isolation of insulin and its further development and refinement during the last decades. We have now moved into a phase of detection drives, with the concept of "prediabetes" assuming greater and greater importance. It is being more and more realized that diabetes is not simply a question of insulin deficiency but of some error in the whole metabolic superstructure of the human organism which still defies definition and elucidation. Althought great progress has been achieved, there are still vast territories to be explored and chartered, especially with regard to the problem of microangiopathies and the further elucidation of insulin antagonists and

antibodies.

I think it is fitting that I should end my essay with the words of one to whom so much is owed by so many, and who has dedicated a lifetime to stimulate work in the field of Diabetes mellitus and the role which insulin plays in the pattern of diabetes, Charles H. Best. In one of his articles, "The future of Diabetes" (1962), he says: "The only acceptable goal is complete knowledge of the situation. Detection drives are very productive and eminently worthwhile, but a comprehensive survey with provision for following all border-line cases for prolonged periods is needed. I state my conviction again that thus far we have not learned how to compensate completely for the loss of physiologic liberation of insulin by periodic injections of it." Although a great deal has been achieved, if the detection of Diabetes mellitus could be carried out in a more widespread fashion, at the earliest possible stage and in a simple manner and if adequate insulin compensation could be provided, the role of insulin might be even more fundamental and the pattern of Diabetes mellitus more profoundly and radically altered.

THE ROLE OF INSULIN IN THE PATTERN OF DIABETES MELLITUS

BIBLIOGRAPHY

Books consulted:

1. Advances in Metabolic Disorders — Levine & Luft. Vol. I.
2. Diseases of Metabolism — Duncan.
3. Biochemical Disorders in Human Disease — Thompson & King.
4. Harrison's Principles of Internal Medicine.
5. Cecil Loebe's Textbook of Medical Treatment.
6. Recent advances in Medicine — Baron, Compston & Dawson.
7. Treatment of Diabetes Mellitus — Joslin, Root, White & Marble.
8. On Diabetes Mellitus — W.P.U. Jackson.
9. Diabetes by 54 authors, edited by R. Williams.
10. Clinical Diabetes Mellitus — Ellenberge & Rifkin.
11. Current therapy 1965 — Conn.
12. Textbook of Medical Treatment — Dunlop, Davidson & Alstead.

Journals consulted:

(A) BRITISH MEDICAL JOURNAL.

1. "A Diabetic Survey. Report of a working party appointed by the College of General Practitioners" June 1962, Page 1497.
2. "Progress in the understanding of treatment of Diabetes Mellitus" by Prof. W.J.H. Butterfield June 1961, Page 1705.

(B) BRITISH MEDICAL BULLETIN

"Insulin Issue" B.M.B. Vol. 16 No. 3 Sept. 1960.

(C) LANCET.

"Diabetes in Malta" by Prof. J.V. Zammit Maempel Dec. 11, 1965, Page I.

(D) DIABETES. JOURNAL OF THE AMERICAN DIABETES ASSO.

1. "Diagnosis of Prediabetes" by W.P.U. Jackson. Jan.-Feb. 1961, Page 33.
2. "The genetics of Diabetes Mellitus" by C.A. Clarke. May-June 1961, Page 175.
3. "Heredity in Diabetes Mellitus" by Arthur G. Steinberg. July-Aug. 1961, Page 269.
4. "I have lived for 40 years the life of a diabetic patient" by R.D. Lawrence. Nov.-Dec. 1961, Page 483.
5. Diabetes Mellitus and Pregnancy" by Court, Futcher & Newton Long. Nov.-Dec. 1961, Page 445.
6. "Concerning the mechanisms of insulin action. Banting Memorial Lecture 1961" by Rachmiel Levine. Nov.-Dec. 1961, Page 421.
7. "The effect of insulin on the liver. A Symposium" by Weinhouse, Madison and deBodo. Jan.-Feb. 1963, Page 1.
8. "Clinical limpact of insulin" by R. Harold. Jan.-Feb. 1963, Page 31.
9. "Diabetic Microangiopathy" by J.M.B. Bloodworth. March-April 1963, Page 99.
10. "Harmonal factors of Diabetic ketosis — Banting Memorial Lecture 1963" by B.A. Houssay. Nov.-Dec. 1963, Page 481.
11. "Diabetic Microangiapthy" by S.I. Zacks. Jan-F.eb. 1964, Page 90.
12. 'The homeostasis of 'Blood Sugar' by F.C. McClean. March-April 1964, Page 198.
13. "The metabolism of insulin" by Arthur Mirsky, May-June 1964, Page 225.
14. "Synalbumin insulin antagonism" by J. Vallance Owen. May-June 1964, Page 241.
15. "The present status of insulin antagonists in plasma" by Yalow & Berson. May-June 1964, Page 247.
16. "The peripheral nervous system in Diabetes Mellitus" by S. Locke May-June 1964, Page 307.
17. "Insulin & Protein Metabolism" by F.D.W. Lukens. Sept-Oct. 1964, Page 451.
18. "Diabetic Neuropathy: a metabolic or a vascular disease?" by J. Perart. Jan. 1965, Page 1.
19. "Vascular disease in diabetes" by Arthur Colwell. Feb. 1965, Page 110.
20. "Reversal of retinal vacsular changes in diabetes" by Dollery & Oakley. March 1965, Page 121.
21. "Diabetes in 1964. A World Survey" by P. Entmacher. April 1965 Page 212.
22. "Neurological disorders of Diabetes Mellitus" by Owen Colby. August 1965, Page 516.
23. "Banting Memorial Lecture 1965" Sept. 1965, Page 549.

(E) JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM.

"Intestinal factors in the control of insulin secretion" by McIntyre, Holdsworth &Turner. Oct. 1965, No. 10, Page 1317.

(F) PROCEEDINGS OF ROYAL SOCIETY OF MEDICINE.

"Summary of the results of the Bedford Diabetes Survey" by W.J.H. Butterfield. Oct. 24, 1963, Page 196.

(G) POSTGRADUATE MEDICAL JOURNAL.

"Diabetes issue. P.M.J. Vol. 15, No. 403, May 1959.

(H) THE QUARTERLY JOURNAL OF MEDICINE.

"Pituitary ablation for Diabetic Retinopathy" by G.F. Joplin, Russel Fraser D.W. Hill, N.W. Oakley, D.J. Scott & F.H. Doyle. Oct. 1965, Vol. 34, No. 136, Page 443.

(I) THE JOURNAL OF MEDICAL GENETICS.

"Genetic factors in Diabetes Mellitus studied by Oral Glucose Tolerance Tests" by G.S. Thompson, Vol. 2 No. 64. Dec. 1965, Page 221.