INSULIN AND DIABETES.

A GENERAL STATEMENT OF THE PHYSIOLOGICAL AND THERAPEUTIC EFFECTS OF INSULIN.

BY

J. J. R. MACLEOD, M.B., CH.B. ABERD.,
PROFESSOR OF PHYSIOLOGY, TORONTO UNIVERSITY.

Early Investigations.

LÉPINE¹ was the first to suggest that the condition of diabetes which von Mering and Minkowski² had discovered to follow pancreatectomy might be due to the withdrawal of an internal secretion necessary in the normal animal for the complete metabolism of sugar. The subsequent discoveries that extracts of certain ductless glands, such as the adrenals, have marked pharmacological actions, and that administration of extracts of others, such as the thyroid, can relieve the symptoms which are associated with atrophy of that gland, gave indirect support for the view that the pancreas must also yield an internal secretion. This view was also greatly strengthened by the results of experiments in which it could be clearly demonstrated that a subcutaneous graft of pancreatic tissue is capable of preventing diabetes when the remainder of the pancreas is removed. Many attempts were made to obtain direct proof of the presence of this hormone; thus diabetic animals were given extracts of pancreas prepared in various ways, or blood from the pancreatic veins of normal animals was injected into them, but with no definite benefit. The extracts prepared by Starling and Knowlton,³ by Murlin and Kramer,⁴ by Kleiner,⁵ and by E. L. Scott⁶—to mention only a few of the investigators—did not yield results that were considered by these authors to be sufficiently positive to justify an immediate further development of their investigations with a view to using the extracts in the treatment of diabetes in man. The hope that, in spite of these more or less unsatisfactory results, a method would some day be discovered for the preparation of a pancreatic extract capable of alleviating the symptoms of diabetes was sustained by the publication from time to time of researches the results of which could be interpreted only by assuming the presence of a pancreatic hormone. Among the most striking of these investigations may be mentioned those of Clarke,⁷ who discovered that the isolated mammalian heart—kept alive by perfusing its blood vessels with Locke's solution containing glucose—removes sugar from the perfusion fluid much more rapidly when the fluid used had first of all been perfused through the blood vessels of the pancreas than from fresh Locke's solution. In the light of these researches the majority of investigators concluded that a pancreatic hormone must exist, but that it was impossible to detect its presence in pancreatic extracts because of its destruction by the trypsin and other proteolytic enzymes also present in them.

Starting-point of the Recent Advance.

The problem therefore was to circumvent these enzymes, and to Dr. F. G. Banting is due the credit of suggesting that this might be accomplished by taking advantage of the well-known fact that the cells which secrete the digestive enzymes become completely degenerated in some weeks after ligation of the pancreatic ducts, whereas those of the isles of Langerhans, which apparently secrete the antidiabetic hormone, remain more or less intact. He undertook to see whether extracts of the degenerated gland might yield more certain results, and facilities and assistance were afforded him in my department to put this suggestion to the test of experiment. In collaboration with C. H. Best, Banting observed the effects produced by injection of the extracts on the percentage of sugar in the blood, as well as on the total sugar excretion in the 24-hour urine of dogs, rendered diabetic by extirpation of the pancreas.⁸ From practically the very start of the observations a definite lowering of both of these values was observed to occur. The observations were frequently repeated with all necessary controls, and it was further noted that the exhibition of the extract also greatly improved the subjective condition of the animal and markedly prolonged its life. An untreated depancreatized dog seldom lives beyond three weeks—or four at the outside—whereas with the extracts one such animal lived in excellent condition for ten weeks, when it was killed by chloroform. No trace of pancreas could be seen by the naked eye *post mortem*, though a very small nodule of zymogenous cells was found in the walls of the duodenum in a careful microscopical examination.

First Therapeutic Application.

Having shown by these experiments that the pancreas does contain an antidiabetic hormone the next step was to find a method by which an extract containing it could be made from undegenerated gland. Knowing that proteolytic enzymes are almost, if not entirely, absent in the foetal pancreas (Ibrahim), Banting and Best⁹ prepared extracts from this source, and, finding them also to contain the antidiabetic hormone, then proceeded to try whether an active extract could be prepared from the pancreas of the full-grown ox, by using alcohol as the extracting medium so as to circumvent the destructive action of trypsin, as had previously been suggested by E. L. Scott. Satisfactory results were obtained, and it was considered that the time had now arrived to warrant the administration of the extract to a diabetic patient. This was done in the case of a boy of 14 years suffering from the usual severe type that is so common at this age. The percentage of blood sugar was reduced by about 25 per cent. This may be considered to mark the close of the first and fundamental step in the discovery of insulin, the name which, following Sir E. A. Schafer's suggestion, has been given to the internal secretion of the pancreas.

Preparation of Insulin.

The presence of a hormone capable of alleviating the symptoms of diabetes both in laboratory animals and in man having been definitely established, it remained to devise suitable methods for the preparation in quantity of an extract containing it and to investigate thoroughly the extent and nature of its physiological and therapeutic actions.

Since the extract used by Banting and Best in the clinical observation referred to was found to cause a certain degree of local irritation, it was necessary, before further clinical trials could be undertaken, to prepare it in purer form. This was successfully accomplished by Dr. J. B. Collip by a method of fractional precipitation with alcohol, the principle of which is described elsewhere,¹¹ and which in all essential details is the one now used for the preparation of insulin in larger quantity. At this stage of the work it also became necessary to find some simple, readily available laboratory method for testing the potency of the preparations of insulin. Without some method of physiological assay it would have been extremely difficult to develop a satisfactory method of preparation on a large scale, because the potency of each precipitate and filtrate in the process of fractional precipitation of the crude extracts had to be tested before the next step in the preparation was taken.

Pharmacological Assay.

Moreover, a method of pharmacological dosage had to be devised to serve as a guide for the clinical dosage. These requirements were met by the discovery that insulin lowers the percentage of blood sugar in normal rabbits.¹² The rate of development, as well as the degree of this hypoglycaemia, depends somewhat on the method of injection (whether subcutaneous or intravenous), on the state of nutrition of the animal, and, of course, on the body weight; but we have found it satisfactory to consider as one unit the amount of insulin which, on subcutaneous injection, can lower the percentage of blood sugar to 0.045 within four hours in a rabbit weighing about 2 kg. from which food has been withheld for sixteen to twenty-four hours. The final solutions of insulin prepared in the University for clinical use are usually concentrated, so that 1 c.cm. contains one unit. The reason why 0.045 was chosen as the percentage to which the sugar should become lowered is that at this level, almost without fail, the animal develops highly characteristic symptoms consisting of violent convulsive seizures with intervals of coma. These symptoms finally terminate in death, but if the animal be injected subcutaneously with dextrose (gram per kilogram) it immediately recovers and usually remains without further symptoms; occasionally a relapse may occur, which, however, can again readily be antidoted by dextrose. No other sugar has the same effect, although a temporary alleviation of the symptoms has sometimes been observed after injections of galactose and laevulose.

Associated Effects of Insulin.

These discoveries, to which Dr. Collip contributed much, opened the way to more extended physiological and clinical investigations. E. C. Noble, J. Hepburn, and J. K. Latchford joined in the physiological investigations, and by excellent team work it was possible in a comparatively short time to

demonstrate many other effects of insulin, a few of which may be briefly alluded to here because of their importance in showing the fundamental nature of its action.

The first step was to extend the investigations of Banting and Best on depancreatized dogs so as to include observations of the respiratory quotient, and the urinary excretion of acetone bodies.¹¹ The former of these is a ratio expressing the relationships of the amount of CO₂ excreted and of O₂ retained by the animal, and it is a reliable qualitative index of the type of metabolism, being of the value of about 1 when carbohydrates are the chief foodstuff undergoing metabolism, 0.7 for fats, and 0.8 for proteins. When diabetes is of the severest type, as in dogs after the removal of the pancreas, the quotient often falls below 0.7, and it is not raised to any significant degree when carbohydrate foods are given. This was found to be the case in the depancreatized dogs used in these investigations, but when insulin was given along with the sugar the quotient immediately rose decidedly, indicating that the power to metabolize carbohydrate had been restored.

Effect on Excretion of Acetone Bodies.

The excretion of acetone bodies in a diabetic dog is not relatively so large as in severe cases of diabetes in man, and it is somewhat irregular. When insulin was given, however, the acetone bodies entirely disappeared from the urine and remained absent so long as the administration was maintained.

Effect on Fat Metabolism.

Very significant results were obtained by comparison of the percentage amounts of glycogen and of fat in the liver and heart in untreated and insulin-treated diabetic animals.¹¹ When no insulin was given the glycogen content of the liver was found to be very low—not more than 1 per cent.—whereas that in the heart was decidedly above the normal; after insulin (and sugar) the percentage of glycogen in the liver rose to the exceptionally high value of over 12 per cent., and there was a decided fall in the percentage in the heart. The fat content of the liver fell from about 12 per cent. in untreated animals to below 5 in those treated for two days with insulin, and there was also a decided diminution in the fat content of the blood. These observations indicate that insulin, possibly because of its influence primarily on carbohydrate metabolism, also affects that of fat.

Influence on Carbohydrate Metabolism.

In the long series of chemical processes which enter into the metabolism of the carbohydrates there are only two products which are sufficiently stable to permit of quantitative determination: these are the sugar of the blood and the glycogen of the liver. In diabetes the former increases and the latter decreases, and insulin restores them to, or beyond, their normal levels. Taking this along with the fact that oxidation of the glucose molecule, as revealed by the respiratory quotient, fails to occur in diabetes, but does occur, as in a normal animal, when insulin is given, it is evident that this hormone must be an essential factor in the regulation of carbohydrate metabolism. Not only, however, does insulin remove the symptoms of diabetes due to absence of insulin from the body, but it also prevents the development of the hyperglycaemia, and presumably of the glycosuria, which can be induced by other experimental means.¹³ The best known of these are puncture of the floor of the fourth ventricle of the medulla, subcutaneous injections of epinephrin, and the various forms of asphyxia, including ether anaesthesia. In rabbits previously fed abundantly with carbohydrate rich foods so as to ensure a high percentage of glycogen in the liver, none of these experimental procedures was found to cause more than a slight increase in the blood sugar in animals previously injected with insulin. The only one of them which could cause any decided degree of hyperglycaemia after insulin was that due to excessive doses of epinephrin, and it is possible that a method for the precise dosage of insulin based on its power to antidote known quantities of epinephrin may be evolved. That insulin almost entirely prevents the development of hyperglycaemia in ether anaesthesia has an evident practical application in surgical practice.

Effect on Cardiac Nutrition.

As to the mechanism of the action of insulin we are as yet in the dark. One other fact of great importance may, however, be mentioned—namely, that it increases the rate of

sugar consumption by the isolated mammalian heart.¹⁴ These observations were made on the hearts of rabbits. Immediately after killing the animal the heart was excised and perfused through the aorta with Locke's solution containing about 0.2 per cent. of glucose. From time to time samples of this fluid were removed for determination of the percentage of reducing power. When no insulin was added to the perfusion fluid the average rate of disappearance of sugar was 0.8 mg. per gram of heart per hour; when insulin was added to the fluid the average was over 3 mg. It was not possible to determine whether the more rapid disappearance of sugar under insulin was due to its increased combustion or to its being deposited as glycogen in the heart. This it will be possible to determine only by observing the respiratory quotient in a heart-lung preparation, such as has been used by Starling and his collaborators.

Mode of Action of Insulin.

This observation on the heart would seem to narrow down the locus of action of insulin to the muscular tissue, and we therefore hoped to find that we could demonstrate that addition of insulin would accelerate the rate of disappearance of glucose from incubated (Buchner) extracts of muscle. So far, however, the results have been entirely negative, although with Dr. G. S. Eadie's assistance we have repeated them in various modifications of the experimental conditions. Neither does insulin have an influence on the rate of disappearance of glucose from drawn blood (glycolysis), nor on the rate at which glycogen is broken down in incubated pieces of liver. The object of doing these experiments is not only that some light might be thrown on the mechanism of action of insulin, but also that some *in vitro* method might be discovered for measurement of the potency of insulin.

Insulin in Diabetes Mellitus.

The effects of insulin in removing the cardinal symptoms of diabetes in depancreatized dogs, as well as those observed on normal rabbits, have been found also to follow its administration in the disease, diabetes mellitus, in man. Every detail of the therapeutic effects of this hormone was definitely forecasted by animal experimentation—the effect on the blood sugar and on the urinary sugar, the diminution of acetone bodies (to which the incidence of fatal coma is related), the prolongation of life, the raising of the power to metabolize sugar—and it was not until this had been established beyond doubt in the laboratory that insulin was given in the wards. Without the experimental demonstration of its effects no physician would have been justified in attempting the therapeutic investigation of insulin. Had he attempted to do so unguided by the results of observations on rabbits, it is almost certain that sooner or later an overdose would have been given, causing convulsions (not unlike those of diabetic coma itself) for which there would have been no indication as to what remedy should be used. The experiments on rabbits, however, showed clearly that these alarming symptoms are immediately antidoted by injection of glucose, and it is of interest that in one or two patients in whom there have been premonitory indications of their possible occurrence exhibition of glucose has immediately and permanently removed them.

A preliminary paper on the principal clinical effects was published in March by Banting, Best, Collip, Campbell, and Fletcher,¹⁵ and since that date a sufficient number of cases of all degrees of the disease have now been observed—partly in the clinic of Dr. Banting at the Christie Street Hospital of the Soldiers' Civil Re-establishment in Toronto, partly in the clinic of Professor Duncan Graham in the Toronto General Hospital, and partly in certain diabetic clinics in the United States—to justify the statement that when insulin is administered subcutaneously in adequate dosage it is capable, within a remarkably short time, of removing the cardinal symptoms of the disease for a period of several hours. To suppress the symptoms permanently, however, the injections must be repeated, the practice at present being twice daily. So long as the administration is maintained the patient is able to assimilate much more carbohydrate than previously, and he gains weight and with it both physical and mental vigour, so that the despondency and apathy that are so prominent in these cases disappear. Whether the diabetic condition is in any degree cured by the rest which is given to the damaged pancreatic function by insulin cannot as yet be stated. It is perhaps in the adolescent forms of the disease that these results have been most marked, but there

can be no doubt that when insulin comes to be more available its exhibition along with intelligent control of diet will have the same beneficial results in all the serious forms of the disease. In cases of threatened coma its value is undoubted, and this is also the case when it is used as a precautionary measure against post-operative risk in surgical practice.

Until the clinical effects have been more thoroughly investigated it would be out of place here to discuss in further detail the therapeutic value of insulin.

Preparation on the Large Scale.

It remains to explain the steps that have been taken by the University of Toronto to safeguard the production, standardization and distribution of insulin. Realizing that the publication of the method of its preparation might be followed by an uncontrolled commercial exploitation of insulin, with the consequence of its improper use in general practice, the chief originators of the method generously offered to apply for patents covering its production, and to hand these over to the University provided this body would assume the function of licensing approved manufacturers to produce and sell it at a reasonable cost. Patents have therefore been applied for in Canada, the United States, Great Britain, and other countries, and the University has appointed a committee, composed partly of those who have participated in the investigations and partly of members of the Board of Governors, to advise it with regard to the conditions under which licences should be granted.

As a first step this committee recommended that licences should not be freely granted until every possible detail of the manufacture on a large scale of a preparation of known and constant potency had been thoroughly worked out. This step was taken because of the unexpected difficulties which were encountered when large scale production was attempted in the Connaught Antitoxin Laboratories of the University. It was found that whereas non-irritating preparations of insulin of constant potency could readily be obtained by Collip's procedure in the laboratory, the yields were for some time extremely unsatisfactory when a large scale production was attempted.

Protection from Commercial Exploitation.

After these difficulties had been overcome it was decided still further to expand the production by collaborating with some firm engaged in the manufacture of extracts from slaughterhouse materials. This was done under a special agreement by which the University undertook not to issue licences to other firms until the lapse of a period during which the large scale method could be carried out so as to yield the maximal output of preparation of standard and uniform potency both when assayed by the rabbit test and when tested therapeutically on diabetic patients. For the clinical assays this firm agreed to distribute a sufficient number of free samples of their preparation to various physicians specializing in the treatment of diabetes in properly controlled clinics. This collaboration between the committee of the University and a firm in the United States is proceeding at the present time, and the physicians receiving insulin are reporting their experiences as to its potency and value. Whenever it is certain that a thoroughly reliable large scale method for a product of constant potency has been worked out the special agreement between the University and the collaborating firm will terminate, and the University will issue licences to other approved firms.

Realizing that it could not exercise proper supervision over the production of insulin in more countries than Canada and the United States, the University has offered to the Medical Research Council the assignment of the patents applied for in Great Britain for the purpose of safeguarding the production and standardization of insulin in that country.

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Remarks

ON THE

SURGERY OF THE PITUITARY GLAND.*

BY

A. J. WALTON, M.S., F.R.C.S.,

SURGEON, LONDON HOSPITAL.

[With Special Plate.]

EVEN in adult life the two portions of the pituitary gland remain distinct, and each appears to have a very different function. Their somewhat complicated development will therefore have to be briefly considered before discussing the manifestations of disease of the gland.

DEVELOPMENT.

In the adult the pituitary gland forms a small oval structure, which is somewhat flattened from above downwards, and fills the pituitary fossa of the sphenoid bone. Its longest diameter, which usually lies transversely, is about half an inch. On section it can be seen to be distinctly formed of two parts, an anterior and a posterior. The anterior portion, often described as the epithelial or glandular part, is in man considerably the larger, and forms about two-thirds of the total gland. It is immediately in front of, and in part envelops, the posterior lobe, which may be regarded as being embedded in its posterior surface. Blair Bell has shown that this position is largely dependent upon the shape of the fossa, for in animals with a deep fossa, such as the cat, the pars nervosa lies posteriorly; while in dogs, which have a shallow fossa, it is placed superior to the glandular part. The glandular portion is developed from the stomadeum at a very early age from a recess, known as Rathke's pouch, which grows upwards from the junction of the pharyngeal membrane with the roof of the primitive stomadeum, and which, according to Bell, may be visible even in a foetus of 2.5 mm. Such a method of development is remarkably constant within the vertebrate series, and may be found in some form even as far back as the Cyclostomata. The pouch so formed passes upwards in front of the notochord and between the trabeculae cranii to the base of the brain. The neck of the sac becomes constricted, but the upper portion dilates to form what is known as the pituitary sac. Later, as the sphenoid develops, the stalk becomes constricted and disappears, so that the pituitary sac is entirely shut off from the stomadeum. The position of this stalk is usually regarded as lying between the pre-sphenoid and post-sphenoid, but Cope has shown that it traverses the anterior part of the post-sphenoid. Although the stalk by these means is as a rule entirely destroyed, Duffy has shown that certain squamous-celled tumours which arise in or about the pituitary probably originate in the remnants of this stalk, which may persist in the post-sphenoid or beneath the capsule of the anterior lobe. Erdheim has reported a case of acromegaly in which a tumour formed of cells resembling those of the anterior lobe was imprisoned in the posterior part of the sphenoid. The pituitary sac thus cut off from the primitive pharynx undergoes very considerable proliferation. It becomes more or less triradiate in outline, and later gives off a large number of diverticula, so that it comes to have a glandular appearance.

The fully developed anterior lobe comes therefore to be composed of well-defined epithelial cells supported by a framework of fine connective tissue and showing large blood vessels and sinuses. Not uncommonly the cells are arranged in the form of small vesicles containing a granular-like material somewhat resembling colloid. The work of Bell and Cushing, among others, shows that there is a very great variation in the appearance of this portion of the gland in different physiological and pathological states. It can always be determined, however, that there are two main types of cells: one of these stains well and is spoken of as the chromophil cell, which may be either basophil or acidophil; the other stains poorly, and is spoken of as the neutrophil or chromophobe cell.

The anterior lobe appears to become larger and more active in certain conditions—such as during pregnancy, after

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