DIABETES AND INSULIN.

Nobel Lecture delivered at Stockholm on September 15th 1925

by

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Gentlemen:

I very deeply appreciate the honour which you have conferred upon me in awarding the Nobel prize for 1923 to me and Professor J. J. R. Macleod. — I am fully aware of the responsibility which rests upon me to deliver an address in which certain aspects of the work on insulin may be placed before you. This I propose to do today and I regret that an earlier opportunity has not been afforded me of satisfying this obligation.

DIABETES AND INSULIN.

Since von Mering and Minkowski proved that removal of the pancreas produced severe and fatal diabetes in dogs, physiologists and clinicians have frequently endeavored to obtain from the pancreas an internal secretion which would be of value in the treatment of diabetes mellitus. Beginning with Minkowski himself many observers tried various forms of extracts of the pancreas. Among the extractives used were water, saline, alcohol and glycerin. The extracts thus obtained were administered by mouth, subcutaneously, intravenously or by rectum, both to experimental animals and humans suffering from diabetes. Little or no improvement was obtained and any favorable results were overshadowed by their toxic effects. In 1908, Zuelzer tried alcoholic extracts on six cases of diabetes mellitus and obtained favorable results, one case of severe diabetes becoming sugar free. His extracts were then tried by Forschbach in Minkowski's clinic with less favorable results, and the investigation was abandoned by this group of workers Rennie found that the islet cells existed separate from the acinar cells in

certain boney fishes and in conjunction with Fraser extracts of the principal islet cells were tried both on animals and on the human. Their results, however, were not sufficiently convincing to warrant clinical application. The problem of the extraction of the antidiabetic principle from the pancreas was then taken up for the most part by physiologists among whom were Scott, Paulesco, Kleiner and Murlin.

While these efforts were being made by the physiologists valuable knowledge was being gained on carbohydrate metabolism. Lewis and Benedict, Folin and Wu, Schaffer and Hartman, and Ivar Bang had elaborated methods whereby the percentage of sugar in a small sample of blood might be accurately estimated. At the same time a vast amount of knowledge was accumulating on basal metabolism. Special attention was being given to the relative importance of the various foodstuffs, and emphasis was being put on dietetic treatment of diabetes. Guelpa, Van Nordam, Allen, Joslin and Woodyatt, had elaborated systems of diabetic diet.

On October 30th, 1920, I was attracted by an article by Moses Baron, in which he pointed out the similarity between the degenerative changes in the acinus cells of the pancreas following experimental ligation of the duct, and the changes following blockage of the duct with gall-stones. Having read this article the idea presented itself that by ligating the duct and allowing time for the degeneration of the acinus cells, a means might be provided for obtaining an extract of the islet cells free from the destroying influence of trypsin and other pancreatic enzymes.

On April 14th, 1921, I began working on this idea in the Physiological Laboratory of the University of Toronto. Professor Macleod allotted me Dr. Charles Best as an associate. Our first step was to tie the pancreatic ducts in a number of dogs. At the end of seven weeks these dogs were chloroformed. The pancreas of each dog was removed and all were found to be shrivelled, fibrotic, and about one-third the original size. Histological examination showed that there were no healthy acinus cells. This material was cut into small pieces, ground with sand, and extracted with normal saline. This extract was tested on a dog rendered diabetic by the removal of the pancreas. Following the intravenous injection the blood sugars of the depancreatized dogs were reduced to a normal or subnormal level, and the urine became sugar free. There was a marked improvement in the general clinical condition as evidenced by the fact that the animals became stronger and more lively, the broken down wounds healed more kindly, and the life of the animal was undoubtedly prolonged.

The beneficial results obtained from this first type of extract substantiated the view that trypsin destroyed the antidiabetic principle and suggested the idea that by getting rid of the trypsin an active extract might be obtained. The second type of extract was made from the pancreas of dogs in which acinus cells had been exhausted of trypsin by the long continued injection of secretin. Although many of the extracts made in this manner produced marked lowering of blood sugar and improvement in the general clinical condition it was not always possible to completely exhaust the gland consequently toxic effects frequently resulted.

The third type of extract used in this series of experiments was made from the pancreas of foetal calves of less than four months development. Laguesse had found that the pancreas of new-born contained comparatively more islet cells than the pancreas of the adult. Since other glands of internal secretion are known to contain their active principle as soon as they are differentiated in their embryological development, it ocurred to me that trypsin might not be present since it is not used till after the birth of the animal. Later I found that Ibrahim had shown that trypsin is not present till seven or eight months of intrauterine development. Foetal extracts could be prepared in a much more concentrated solution than the former two varieties of extract. It produced marked lowering of blood sugar, urine became sugar free and there was marked clinical improvement. Its greatest value however was that the abundance in which it could be obtained enabled us to investigate its chemical extraction.

Up to this time saline had been used as an extractive. We now found that alcohol slightly acidified extracted the active principle, and by applying this method of extraction to the whole adult beef pancreas active extracts comparatively free from toxic properties were obtained.

Since all large scale production methods for the preparation of Insulin today have the acid-alcohol extraction as the first step in the process it may be well to elaborate on the methods of preparation at this stage. Insulin was prepared by the extraction of fresh glands with faintly acid alcohol. The concentration of alcohol in the original experiments varied from 40 to 60 per cent. The alcoholic solution of pancreas was filtered and the filtrate concentrated by evaporation of the alcohol and water in vacuo or in a warm air current. Lipoid material was removed by extracting the residue with toluene or ether. The resulting product was the original whole gland extract. We were able to show that the active material contained in this extract was practically insoluble in 95 % alcohol.

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The extracts prepared in this way were tried on depancreatized dogs and in all cases the blood sugar was lowered. In one early case hypoglycaemic level was reached and the dog died from what we now know to be a hypoglycaemic reaction.

It had been known that depancreatized dogs were unable to store glycogen in the liver, and that glycogen disappears in three or four days after pancreatectomy. We found that by the administration of glucose and extract the diabetic dog was enabled to store as much as 8 % to 12 % glycogen. Diabetic dogs seldom live more than 12 to 14 days. But with the daily administration of this whole gland extract we were able to keep a depancreatized dog alive and healthy for ten weeks. At the end of this time the dog was chloroformed and a careful autopsy failed to reveal any islet tissue.

The extract at this time was sufficiently purified to be tested on three cases of diabetes mellitus in the wards of the Toronto General Hospital. There was a marked reduction in blood sugar and the urine was rendered sugar free. However the high protein content rendered the continuous use undesirable, due to formation of sterile abscesses.

At this stage in the investigation, February 1923, Professor Macleod abandoned his work on anoxaemia and turned his whole laboratory staff on the investigation of the physiological properties of what is now known as Insulin.

Dr. Collip took up the biochemical purification of the active principle and ran the scale of fractional precipitation 70 % to 95 % alcohol and succeeded in obtaining a more improved end product. But unfortunately his method was not applicable to large scale production. Dr. Best then took up the large scale production and contributed greatly to the establishment of the principles of production and purification. This work was carried out on the Connaught Laboratories under Prof. Fitzgerald who is kind enough to be here today.

It had been found that the final product obtained by the earlier methods was not sufficiently pure for prolonged clinical use and efforts were made to secure a better product. The benzoic acid method of Maloney and Findlay which depends upon the fact that Insulin is absorbed from watery solutions by benzoic acid was successfully used in Connaught Laboratories for several months.

Professer Shaffer of Washington University, St. Louis, and his collaborators, Somogyi and Doisy, introduced a method of purification which

is known as the isoelectric process. This method depends upon the fact that if a watery solution of Insulin is adjusted to approximately P_H 5 a precipitate settles out which contains much of the potent material and relatively few impurities. Dudley has found that Insulin was precipitated from water solutions by picric acid and he made use of this fact to devise a very ingenious method for the purification of the active material.

Best and Scott who are responsible for the preparation of Insulin in the Insulin Division of the Connaught Laboratories have tested all the available methods and have appropriated certain details from many of these, several new procedures which have been found advantageous have been introduced by them. The yield of Insulin obtained by Best and Scott at the Connaught Laboratories, by a preliminary extraction with dilute sulphuric acid followed by alcohol, is 1,800 to 2.200 units per kg of pancreas.

The present method of preparation is as follows. The beef or pork pancreas is finely minced in a large grinder and the minced material is then treated with 5 c. c. of concentrated sulphuric acid, appropriately diluted, per pound of glands. The mixture is stirred for a period of three or four hours and 95 per cent alcohol is added until the concentration of alcohol is 60 to 70 per cent. Two extractions of the glands are made. The solid material is then partially removed by centrifuging the mixture and the solution is further clarified by filtering through paper. The filtrate is practically neutralized with NaOH. The clear filtrate is concentrated in vacuo to about 1/15 of its original volume. The concentrate is then heated to 50° C which results in the separation of lipoid and other materials, which are removed by filtration. Ammonium sulphate (37 gms. per 100 c. c.) is then added to the concentrate and a protein material containing all the Insulin floats to the top of the liquid. The precipitate is skimmed off and dissolved in hot acid alcohol. When the precipitate has completely dissolved 10 volumes of warm alcohol are added. The solution is then neutralized with NaOH and cooled to room temperature, and kept in a refrigerator at 5° C for 2 days. At the end of this time the dark-coloured supernatant alcohol is decanted off. The alcohol contains practically no potency. The precipitate is dried in vacuo to remove all trace of the alcohol. It is then dissolved in acid water, in which it is readily soluble. The solution is made alkaline with NaOH to P_H 7.3 to 7.5. At this alkalinity a dark-coloured precipitate settles out, and is immediately centrifuged off. This precipitate is washed once or twice with alkaline water of P_H 9.0 and the washings are added to the main liquid. It is important

that this process be carried out fairly quickly as Insulin is destroyed in alkaline solution. The acidity is adjusted to P_H 5.0 and a white precipitate readily settles out. Tricresol is added to a concentration of 0.3 % in order to assist in the isoelectric precipitation and to act as a preservative. After standing one week in the ice chest the supernatant liquid is decanted off and the resultant liquid is removed by centrifuging. The precipitate is then dissolved in a small quantity of acid water. A second isoelectric precipitation is carried out by adjusting the acidity to a P_H of approximately 5.0. After standing over night the resultant precipitate is removed by centrifuging. The precipitate, which contains the active principle in a comparatively pure form, is dissolved in acid water and the hydrogen ion concentration adjusted to P_H 2.5. The material is carefully tested to determine the potency and is then diluted to the desired strength of 10, 20, 40, or 80 units per c. c. Tricresol is added to secure a concentration of 0.1 per cent. Sufficient sodium chloride is added to make the solution isotonic. The Insulin solution is passed through a Mandler filter. After passing through the filter the Insulin is retested carefully to determine its potency. There is practically no loss in berkefelding. The tested Insulin is poured into sterile glass vials with aseptic precautions and the sterility of the final product thoroughly tested by approved methods.

The method of estimating the potency of Insulin solutions is based on the effect that Insulin produces upon the blood sugar of normal animals. Rabbits serve as the test animal. They are starved for twenty-four hours before the administration of Insulin. Their weight should be approximately 2 kg. Insulin is distributed in strengths of 10, 20, 40 and 80 units per c. c. The unit is one-third of the amount of material required to lower the blood sugar of a 2-kg. rabbit which has fasted twenty-four hours from the normal level (0.118 per cent) to 0.045 per cent over a period of five hours. In a moderately severe case of diabetes one unit causes about 2.5 grammes of carbohydrate to be utilized. In earlier and milder cases, as a rule, one unit has a greater effect, accounting for three to five grammes of carbohydrate.

With the improvement in the quality of Insulin, the increased knowledge of its physiological action and the increased quantities at our disposal, we were now prepared for more extensive clinical investigation. In May 1922, a clinic was established in association with Dr. Gilchrist, at Christie Street Hospital for returned soldiers. Following this, a clinic was established in the Toronto General Hospital in association with Drs. Camp-

bell and Fletcher, and at Toronto Hospital for Sick Children in association with Dr. Gladys Boyd. In general the routine followed in all these clinics was as follows.

After a careful history had been taken, the patient was given a complete physical examination. Special attention was directed to the finding of foci of possible infection. The teeth, tonsils, accessory sinuses, chest and digestive system were examined clinically, as well as by X-ray. Special consideration was given to biliary tract infection, constipation and chronic appendicitis. If any source of septic absorption was located it was appropriately treated, since such conditions may lower carbohydrate tolerance. If indicated the eye grounds were examined for a possible diabetic retinitis or neuro-retinitis.

The daily routine urinalysis included the volume of the twenty-four hour specimen, the specific gravity, the reaction, and tests for albumen by heat or nitric acid. The acetone bodies were estimated by means of the Rothera and ferric chloride tests. Sugar determinations were done by means of the Benedict qualitative and quantitative solutions. In addition to the above, the blood sugars were estimated by means of the Schaffer-Hartman method and the respiratory quotients with the Douglas bag and Haldane gas analysis apparatus.

At first the patient continued on the same diet as that previous to his admission to hospital in order to obtain some idea of the severity of his case, and to avoid complications from sudden change of diet. Coma will be discussed separately. On the second or third day he was placed upon a diet the caloric value of which was calculated on his basal requirement. This was determined from Dubois' chart and Aub-Dubois' table. It has been estimated by Marsh, Newburg, and Holly that the body requires two-thirds of a gram of protein per kilogram, (1 kilo = 2.2 pounds), of body weight per day, in order to maintain nitrogenous equilibrium. The remaining calories must be supplied by carbohydrate and fats in a ratio that will prevent the production of ketone bodies.

The patient remained on this basal requirement diet at least a week. During this time, blood sugar was estimated before, and three hours after, breakfast, in order to determine the fasting level and the effect of food. The quantity of sugar excreted was estimated daily, and this amount subtracted from the available carbohydrate ingested gives approximately the utilization. The available carbohydrate includes fifty-eight per cent of the protein, ten per cent of the fat, and the total carbohydrate in the diet.

It may be noted that when a patient was placed upon a diet in which the protein, fat and carbohydrates were balanced, that the amount of sugar excreted soon approached a fairly constant amount, whereas if the diet was not well adjusted to the patient's requirements, there was wide variation in the amounts of sugar excreted.

If a patient became sugar-free and blood sugar normal on a basal requirement diet the caloric intake was gradually increased until sugar appeared in the urine. The tolerance was thus ascertained. If a patient remained sugar-free and had a normal blood sugar when on a diet containing five hundred calories above his basal requirement he was not considered sufficiently severe for Insulin treatment, since five hundred calories over and above the basal requirement are sufficient for daily activities. If, however, he vas unable to metabolize this amount, Insulin treatment was commenced.

Diabetes mellitus is due to a deficiency of the internal secretion of the pancreas. The main principle of treatment is, therefore, to correct this deficiency. If it is found that the patient is unable to keep sugar-free on a diet that is compatible with an active, useful life, sufficient Insulin is administered to meet this requirement.

In severe cases Insulin was administered subcutaneously three times a day, from one-half to three-quarters of an hour before meals. This was done so that the curve of hypoglycaemia produced by the Insulin was superimposed on the curve of hyperglycaemia produced by the meal. In rare cases a small fourth dose was given at bed time to control nocturnal glycosuria. The less severe cases could be satisfactorily treated on a morning and evening dose or a single dose before breakfast.

When the Insulin treatment was established, if sugar was present in the twenty-four hour specimen of urine, the dosage was gradually raised till the patient became sugar-free. If he was not receiving sufficient food for maintenance, diet and dosage of Insulin were gradually raised. If small quantities of urinary sugar persist, it was desirable to find out at what period of the day this was excreted. In order to do this, each specimen in the twenty-four hours was analysed separately. An increase in the dose previous to the appearance of glycosuria will prevent its occurrence.

In severe cases it was found preferable to give the largest dose of Insulin in the morning, and reduced doses throughout the day. For example, a patient may receive fifteen units in the morning, ten units at noon and ten units at night. If three equal doses are given there may be morning

glycosuria and evening hypoglycaemia, whereas the extremes of blood sugar causing these conditions may be prevented by the above distribution.

The effect of the same dosage of extract on different individuals was found to vary considerably. Five patients, whose weights varied from forty-six to sixty-seven kilograms, each received two cubic centimetres of the same lot of Insulin, and in four hours the blood sugars had decreased 0.012 %, 0.044 %, 0.128 %, 0.146 %, and 0.0180 % respectively. It was found, however, that one patient would persistently give marked decreases in blood sugar after Insulin, while in another the fall in blood sugar was persistently less. In our experience, the more marked decreases in blood sugar occurred in the milder cases.

The blood sugars of some of the patients were followed throughout the twenty-four hours and it was found that it was possible to gauge the dosage of Insulin so as to keep the blood sugar within normal limits and still avoid the dangers of hypoglycaemia.

Coincident with the maintenance of the blood sugar at normal level the cardinal symptoms of the disease disappear. The patient loses the irritating thirst and dryness of the mouth and throat, and does not desire the large amounts of fluid with which he had previously tried to combat these symptoms. The lowered fluid intake diminishes the polyuria and from a twenty-four hour excretion of three to five litres the output falls to normal. The appetite which has been voracious is now satisfied with a normal meal, the carbohydrate of which is utilized, and the patient loses the persistent craving for food.

We found that when a patient was given too large a dose of Insulin there was a marked reaction, and the hypoglycaemia which developed gave rise to symtoms which were very similar to those observed in animals. The reaction began in from one and a half to six hours after the patient received the overdose. The average time was three to four hours. The interval varied with the individual, the dosage, and the food ingested. The first warning of hypoglycaemia was an unaccountable anxiety and a feeling of impending trouble associated with restlessness. This was frequently followed by profuse perspiration. The development of this symptom was not affected by atmospheric conditions. It appeared while the patient was in a frosty outside atmosphere, or in a heated room, and was independent of physical or mental activity. At this time there was usually a very great desire for food. No particular foodstuff was desired, but bulk of any kind seemed to give satisfaction. At times the appetite is almost unappeasable.

At this stage of the reaction the patient noticed a certain sensation as of clonic tremor in the muscles of the extremities. This could be controlled at first. Coordination, however, was impaired for the more delicate movements. Coincident with this there was a marked pallor of the skin with a rise in pulse rate to one hundred or one hundred and twenty beats per minute, and a dilatation of the pupils. The blood pressure during this period fell about fifteen to twenty-five millimetres of mercury, and the patient felt faint. The ability to do physical or mental work was greatly impaired. In a severe reaction there was often a considerable degree of aphasia, the patient having to grope for words. The memory for names and figures became quite faulty.

The onset of hypoglycaemic symptoms depends not only on the extent, but also on the rapidity of fall in blood sugar. The level at which symptoms occur is slightly higher in the diabetic with marked hyperglycaemia than in a patient whose blood sugar is normal. When the blood sugar is suddenly reduced from a high level premonitory symptoms may occur with a blood sugar between the normal levels of 0.100 % and 0.080 %, while the more marked symptoms of prostration, perspiration, and incoordination develop between 0.080 % and 0.042 %. As a patient becomes accustomed to a normal blood sugar the threshold of these reactions becomes lower. One patient who formerly had premonitory symptoms of hypoglycaemia at 0.096 % now has no reaction at 0.076 %, but symptoms commence between this level and 0.062 %.

The ingestion of carbohydrate, in the form of orange juice, (four to eight ounces) or of glucose, relieves these symptoms in from one-quarter to one-half hour. If the reaction is severe, or if coma or convulsions occur, epinephrin or intravenous glucose should be given. The former acts in from three to ten minutes, but in order that the symptoms should not recur glucose must be given by mouth as soon as the patient has sufficiently recovered. The patients were warned that when these reactions ocurred they were to obtain carbohydrate immediately.

»Fats only burn in the fire of carbohydrate» The ability of the severe diabetic to burn glucose is markedly impaired, therefore the excess of fat is incompletely oxidized, giving rise to ketone bodies. These appear in the blood and urine as acetone, diacetic and betaoxybutyric acids. Insulin causes increased carbohydrate metabolism, and consequently fats are completely burned. This is substantiated by the fact that acetone and sugar disappear from the urine almost simultaneously following adequate amounts

of Insulin. When Insulin is discontinued in these cases, acetone bodies and sugar reappear in the urine.

Since the Rothera test is exceedingly delicate, (sensitive to 1 part of aceto-acetic acid in 30,000), patients on a high fat diet may be sugar-free and still show traces of acetone bodies. A comparison with the ferric chloride test, (which is sensitive to only 1 part in 7,000) is, therefore, desirable. The persistence of ketone bodies in amounts which can be determined by the ferric chloride test necessitates either an increase in the carbohydrate or a decrease in fat of the diet.

When the production of acetone bodies is more rapid than the excretion they accumulate in the blood, giving rise to air hunger, drowsiness, and coma. The need of Insulin is then imperative. After its administration the utilization of carbohydrate by the body gives complete combustion of the fats. When a patient was admitted to hospital in coma the blood sugar tests and a urinalysis were done as soon as possible. (The urine was obtained by catheterization if necessary.) While these tests were being carried out the large bowel was evacuated with copious enemata. If the blood sugar was high and acetone present in large amounts in the urine, from thirty to fifty units of Insulin were given subcutaneously. Blood and urinary sugar were frequently estimated because of the danger of hypoglycaemia. To prevent this from thirty to fifty grammes of glucose in ten per cent solution were given intravenously. If the patient was profoundly comatose the Insulin was administered intravenously with the glucose.

The patient usually regained consciousness in from three to six hours. From this time on, fluids and glucose were administered by mouth if retained. The patient was urged to take at least two hundred cubic centimetres of fluid per hour. In from eight to ten hours the ketone bodies were markedly reduced. On the following day protein was given every four hours as the white of one egg in two hundred cubic centimetres of orange juice. In two to three days, when ketone bodies had disappeared from the urine, fat was cautiously added, and the patient was slowly raised to a basal requirement diet. He was then treated as an ordinary diabetic. During the period of coma the patient was kept warm and toxic materials eliminated from the bowel by purgation and repeated enemata. A large amount of fluid was given to dilute the toxic bodies and promote their elimination. This was administered intravenously, subcutaneously, or per rectum. If signs of circulatory failure developed these were treated by appropriate stimulation.

Striking results were obtained with the above procedure. However, it was found that the longer the period of untreated coma the more grave was the prognosis and the slower the recovery if it occurred. Cases complicated by severe infection, gangrene, pneumonia, or intestinal intoxication may recover from acidosis and coma, but succumb to the complication.

Marked lipaemia was present in three cases. This disappeared in the course of a week to ten days after the patient was placed on Insulin and on a diet in which the fat was restricted. The urine of one patient became acetone-free while lipaemia persisted.

The severe diabetic, whose ability to burn carbohydrate is markedly impaired, has a persistently low respiratory quotient, from 0.7 to 0.8, which is but little raised by the ingestion of glucose: when glucose and Insulin are given together the respiratory quotient is markedly increased, showing that carbohydrate is being metabolized. The highest values have been obtained when pure glucose was used with Insulin. Less extensive rises have been secured when the patient, while on a mixed diet, received Insulin.

All the patients gained in weight on the additional calories. There was an increase in sexual vigour and there was a greater ability to do mental and physical work. Nearly all of the patients have returned to their former employment, and while still under supervision, they administer their own Insulin and arrange their own diets with satisfactory results.

All diabetics who have not an adequate knowledge of the dietetic treatment of their disease should be admitted to hospital in order that they may receive instruction in the preparation of their calculated and weighed diet — that they may learn the qualitative tests for sugar and acetone in the urine — that their carbohydrate tolerance may be accurately determined; and that the use of Insulin, if required, may be safely instituted. Mild cases, especially if over fifty years of age can be controlled by diet. Cases that cannot be adequately controlled by dietetic treatment alone should be given sufficient Insulin to enable them to attain to a diet on which they may »carry on».

One of the commonest complications of diabetes especially in untreated patients over fifty is gangrene. It is often associated with varying degrees of sclerosis of the leg arteries, which makes it extremely difficult to obtain healing. This may be accomplished by the use of Insulin, but when permanent impairment has occurred it is advisable to amputate. Amputation is also advisable when an infection is so severe that the life of the

patient is in jeopardy. Treatment of these cases is difficult because, due to the infection, there is a marked variation in the daily production of Insulin by their own pancreas. But with careful treatment they can be rendered free from acetone and sugar and their general condition improved. Operation is then performed preferably under nitrous oxide and oxygen anaesthetic. If the blood sugar is maintained normal and acidosis is prevented the wound heals kindly, provided that the amputation has been high enough to assure a good blood supply. For varying periods after the operation the patient remains on Insulin treatment. In nearly all cases at the end of three of four weeks mild hypoglycaemic reactions indicate an overdose of Insulin. It is then necessary to increase the diet or decrease the Insulin. In some cases the tolerance improves sufficiently to warrant the discontinuance of Insulin.

Diabetic patients requiring major operations, such as appendectomy, cholecystectomy and tonsillectomy, or removal of teeth, are first rendered sugar and acetone-free unless the severity of symptoms demand immediate attention. Patients formerly considered bad surgical risks, if given proper dietetic treatment with Insulin may be protected from the acidosis, hyperglycaemia and glycosuria which otherwise usually result from the anaesthetic. In the diabetic, infections such as boils and carbuncles and also intercurrent infections such as bronchitis, influenza, and fevers are favorably influenced by the normal blood sugar and increased metabolism which the administration of Insulin permits. In the diabetic with tuberculosis Insulin allows the administration of proper nourishment to combat the tubercle infection.

During the past year and a half I have not been in active practice but have remained associated with the clinics. I have also kept in personal touch with the first fifteen patients who received Insulin treatment. These patients were all extremely severe diabetics for whom diet had done its best. Of these fifteen patients seven were children under fifteen years. It has been possible through the intelligent co-operation of the parents to continue a proper balance between diet and Insulin dosage and to maintain six of the seven children sugar-free. None of these have had to return to hospital, and all have gained in tolerance and require from one-half to one-third less Insulin than when they first began treatment. They have all gained in height and weight and for the most part have developed into healthy normal children. The one child whose diet and Insulin has not been properly controlled has been back in hospital repeatedly and is

steadily losing in tolerance. Of the remaining eight cases there were four women and three men whose ages ranged from twenty-five to thirty-five years. The weight of the women varied from seventy-four to seventy-nine pounds. Two of the women, although they have gained to normal or overweight and now have no symptoms of disease, have not shown any increase in tolerance, due perhaps, to the fact that they have not kept sugar-free. All the others, both men and women, have been able to reduce their dose of Insulin from two-thirds to one-fifth of the original requirement. The one remaining case was admitted for amputation. She had had diabetes for six years, and at the time of admission her blood sugar was 0.350 % and large amounts of acetone and sugar were being excreted in the urine. She was rendered sugar- and acetone-free by means of Insulin before the operation was performed. Amputation was done at the middle third of the thigh. The stump was entirely healed in three weeks. Within six weeks of her operation Insulin was discontinued and her diet was increased without the return of diabetic symptoms. It is now three years since her operation and she is sugar-free on a liberal diet without Insulin.

It may be of interest to mention a few cases in greater detail to further illustrate the improvement in carbohydrate tolerance following Insulin treatment.

Case 1, male, aged 29 years had suffered from chronic appendicitis. The urine of the patient in December, 1916, was sugar-free. About the middle of March, 1917, ha suddenly developed polyuria, polyphagia and polydipsia, and lost fourteen pounds in weight in a fortnight. There was marked weakness. Urinary sugar was discovered to be as high as eight per cent at this time. On April 4th, the patient was placed on Allen treatment, and slowly regained a tolerance of about two hundred grammes available carbohydrate. He returned to his army duties in September 1917, and was able to carry on uninterruptedly until March, 1919. His tolerance had decreased during this time to about one hundred and fifty grammes. Following discharge from the army in March, 1919, the course of the patient was slowly downhill until October, 1921, when a particularly severe form of influenza shattered his tolerance. Up to this time the patient was maintained practically sugar-free, but following the attack of influenza his tolerance fell to about sixty-six grammes of available carbohydrate. He began to lose weight rapidly. Thirst, hunger and polyuria returned. His strength diminished and, owing to mental and physical lassitude, he found it impossible to continue his work. Glycosuria became persistent and ace-

tone bodies made their appearance and steadily increased. A distinct odour of acetone was at times distinguishable on the patient's breath.

On February 11th, 1922, this patient was taken to the Physiology Department of the University of Toronto, and the respiratory quotient was found to be 0.74, and unchanged by the ingestion of thirty grammes of pure glucose. Then 5 cc. of Insulin were given subcutaneously, and within two hours the patient's respiratory quotient had risen to 0.90. The urine was sugar-free and he had shaken off his mental and physical torpor. Following this experiment the patient did not again receive Insulin until May 15th, as the product was being further improved. Since the latter date, the patient has been constantly on Insulin.

During the first six months of Insulin treatment is was impossible to maintain him sugar-free, although he received about 120 units per day. However, he gained in weight and his clinical condition improved. About January 1923 with the improvement in the quality of Insulin, the patient became sugar-free and has remained sugar-free with the exception of one or two occasions. During the first nine months he required no reduction in the dose of Insulin but since that time on the average of every two months he has had a series of hypoglycaemic reactions which necessitated the reduction of the dose. One exception to this occurred in June 1924 at which time appendectomy was performed following a mild attack of appendicitis. An increased dose was required to maintain him sugar-free during this period. At the present time he requires but 20 units of Insulin, or one-sixth of his original requirement. His diet has been practically constant during the whole period of observation. All symptoms attributable to diabetes have long since disappeared. He has gained twenty-five pounds in weight and apart from the necessity of taking Insulin and controlling his diet he leads an active normal life.

This case is a striking example of the fact that it is only in cases who are maintained sugar-free over long periods of time that an improvement in tolerance is obtained with a consequent reduction in the dose of Insulin.

Case 2, female, age 15 years. In the autumn of 1918 the patient had polydipsia and polyuria and complained of weakness. During the winter she suffered from pains in the legs and back and from insomnia. In March, 1919, these symptoms became more severe. The appetite became excessive and there was some pruritus. The weight by this time had fallen from seventy-five pounds to sixty-two pounds. Glycosuria was discovered

and she was placed under the care of Dr F. M. Allen, to whom we are very much indebted for complete record of the case from April 1919 till August 1922. During this period the diet was controlled so as to maintain the urine free from sugar. Despite this careful dietetic regime the patient's condition became progressively worse.

When she came under my care on August 16th, 1922, the examination showed patient emaciated; skin dry; slight edema of ankles. Hair brittle and thin. Abdomen prominent. Marked weakness. The patient was brought on a stretcher and weighed forty-five pounds. Nothing of note in the respiratory, cardiovascular, digestive or nervous system.

At this time she was receiving a diet of protein 50 gm., fat 71 gm., carbohydrate 20 gm. (919 calories). Insulin treatment was started immediately. At this early stage the unit of Insulin had not been worked out and it is therefore difficult to accurately estimate the dosage she received. The diet was increased daily so that, at the end of two weeks, she was receiving protein 63 gm., fat 208 gm., carbohydrate 97 gm., (2512 calories). This diet was continued up to January 1st, 1923. Insulin was given 15 to 30 minutes before the morning and evening meals. A sufficient amount was given to maintain the urine free of sugar. Each specimen of urine was examined and the dose was increased slightly if traces of sugar appeared. When hypoglycaemia occurred orange juice or glucose candy was given. Between August 16th and January 1st, the urine was sugar free except on ten occasions when traces of sugar appeared, and on two other occasions when less than 2 gm. was excreted. Acetone was absent from the urine.

On this treatment the patient gained rapidly in strength and was soon able to take vigorous exercise. Her weight increased from forty-five to one hundred and five pounds in the first six months. The diet included such foodstuffs as cereals, bread, potato, rice, corn, tapioca, corn starch, and even honey.

At present (June 1925) she is in the best of health and to use her own words »never felt better in all my life«. She has grown four inches and weighs one hundred and thirty-four pounds. Her present diet which is only approximate because she has dispensed with the weighing of food, is 125 gm. carbohydrate, 50 gm. protein, 50 gm. fat. This diet is practically the same as that of December 1922. The Insulin required to maintain her sugar-free has been reduced about one-third.

Dr Gladys Boyd, who is now in charge of the diabetics at the Hospital for Sick Children, Toronto, has been able to follow a number of cases

of children under Insulin treatment. She has estimated the Insulin requirement per 10 gm. of carbohydrate in a number of cases and in general her results show a decided increase in tolerance in all cases in which glycosuria and hyperglycaemia are adequately controlled. To illustrate — Case 1, which required 6.9 units per 10 gm. carbohydrate in March 1923, only required 2.6 units in January 1924. Case 2, which required 7.8 units per 10 gm. in January 1925, in June 1925 required only 2.8 units. Case 3, which required 6.5 units per 10 gm. in April 1922, required only 3.7 units in January 1925.

From a review of the work Dr. Boyd has found that all the patients had had hyperglycaemia or even glycosuria at times, but if such occurrences were only transitory and infrequent, improvement in tolerance occurred. Even short periods of rest to the pancreas by means of balanced diet and Insulin resulted in improvement in tolerance. Two of our earliest cases, Fanny Z. and Elsie N. are the only exceptions to this rule. Fanny is to all appearances in the best of health with a blood sugar of 0.3 % to 0.4 %. She has been admitted in coma four times. During her stay in hospital she improves but does as she chooses on discharge. Her tolerance is becoming less all the time. Elsie keeps in touch with us but is looked after by another physician. He purposely allows her to have glycosuria at night. She is fine physically, but requires much more Insulin than formerly.

Dr. Boyd has also found that in those cases who can handle sufficient food without Insulin, although the disease has been kept under control there has not been such striking increase in tolerance.

The best evidence that there is regeneration of the pancreas with Insulin treatment is provided by Drs. Boyd and Robinson. The following is the case reported by them.

Clinical History: B. N. white, male, aged 9 years. Family History: Father and one maternal uncle have diabetes. Diabetes diagnosed in this child when he was two years old. He was placed on a suitable Allen diet, which was strictly adhered to, and for a time did well except for recurrent attacks of dysentery, which lowered his tolerance. Failure to gain in stature or weight in any way commensurate with his age was noted and the general condition became worse each year until he was more or less a chronic invalid with increasingly frequent attacks of acidosis during the last year before starting Insulin.

He was admitted to the Hospital for Sick Children, Toronto, the end

of December 1922. At this time he was an emaciated dwarf, more or less drowsy and unhappy. His weight was thirty pounds, and his height thirty-nine inches. His tolerance to carbohydrate had decreased until he was unable to utilize 15 gm. of such food. Insulin treatment was started at once and his diet increased to a diet suitable for a boy of his age. Sufficient Insulin was given to keep him sugar-free and his blood sugar normal. He was discharged on an adequate diet plus Insulin. Progress both in general condition and in improvement of pancreatic function was steady. His tolerance to carbohydrate trebled in the year as shown either by the fact that 30 units of Insulin controlled the disease as adequately as 90 units a year before, or, stated in another way, without Insulin he could now handle 54 gm. carbohydrate instead of 15. From a chronic invalid in 1922 he became »the leader of the gang» in 1923. He was killed by fracturing his skull when sleigh riding. He lived for about three hours after receiving the injury and an immediate post-mortem examination was made. The pancreas was removed within thirty minutes of death.

From this clinical history one might expect the pancreas to show marked degeneration. However, on section there was little sign of degeneration, but on the other hand there was strong evidence to support the view of active regeneration both of acinar and islet tissue. These regenerative changes were more marked in the periphery and smaller lobules of the pancreas than in the central area.

The acinar cells were found to be actively proliferating in cords and clusters forming small lobules in some areas and were in close association with newly formed functioning ducts.

The islets were greatly increased in number, particularly in the periphery, there being about four times as many per field as in the central area. These cells were large but might be overlooked with an ordinary stain. However, they could be identified as islet cells by Bowie's special granule-stain. This stain also demonstrated that these cells were almost entirely beta cells and were probably concerned in the increased carbohydrate tolerance. On the other hand those islets in the central areas showed an increased number of cells all in an active state of nutrition, but closely packed together. The special stain showed a normal ratio of alpha and beta cells.

These sections were studied by Bensley, Opie, Allen and others who concurred in the opinion of Drs. Boyd and Robinson.

Dr. F. M. Allen, Morristown N. J., after using Insulin for three years

states as his belief, »That there has been improvement of tolerance in some cases beyond what was possible without Insulin». »This observation is trustworthy only in cases where prolonged strict control of symptoms by diet was previously employed. On the other hand the marked increase of tolerance is limited to a minority of cases and has not proved to be continuous in any of them. In other words the improvement always stops short of a cure. There is certainly no decline of tolerance with the passage of time provided the case is kept under proper control.»

This summary is the belief of the most conservative of the outstanding clinicians in the United States engaged in diabetic work on a large scale.

Dr E. P. Joslin, Boston, Mass., who has one of the largest diabetic clinics in the world, has also found that, »The diabetic who is able to reduce his Insulin is the diabetic who is absolutely faithful to diet and restricts gain in weight to a moderate degree.»

Joslin and his associates have carefully analysed the gain in weight and height of their thirty-two diabetic children under fifteen years of age. Their conclusions are:

1. The gain in weight of the diabetic child treated with Insulin resembles that of the normal child, but the diabetic child is still under weight for his age, though often not for his height.

2. The increase in height of the diabetic child treated with Insulin, though occasionally normal, is usually below that of the normal child. So far he has not grown tall like the normal child, either at the expense of growing thin or while being well nourished.

Of the one hundred and thirty children treated with Insulin one hundred and twenty are still living, while of the one hundred and sixty-four who did not receive Insulin there are one hundred and fifty-two dead. Of the one hundred and twenty still living forty per cent have either not increased or have actually decreased their Insulin. Dr. Joslin believes that if the sixty per cent who have had to increase their Insulin had received similar treatment they too would have been able to reduce their Insulin.

Sixteen children under ten years of age who have taken Insulin under Dr. Joslin's care for an average of two years are all alive and now their duration of life is more than three times the duration of life of diabetic children of similar age treated by Dr. Joslin prior to 1915.

Regardless of the severity of the disease, it has been found that by carefully adjusting the *diet and the dose of Insulin, all patients may be main-*

tained sugar-free. Since this is possible, it is to be strongly advocated, because we have abundant evidence for the belief that there is *regeneration of the islet cells of the pancreas* when the strain thrown upon them by a high blood sugar is relieved. The *increase in tolerance* is evidenced by the *decreasing dosage of artificially administered Insulin. In fact, in some moderately severe cases*, the tolerance has increased sufficiently that they no longer require Insulin.

Diabetes mellitus may be considered fundamentally as a *disordered metabolism, primarily of carbohydrates*, and *secondarily of protein and fat.* It is indisputably proven that for normal metabolism of carbohydrate in the body, adequate amounts of Insulin are essential. It follows, therefore, that the treatment consists in giving just sufficient Insulin to make up for the deficiency in the patient's pancreas.

Insulin enables the severe diabetic to burn carbohydrate as shown by the rise in the respiratory quotient following the administration of glucose and Insulin. It permits glucose to be stored as glycogen in the liver for future use. The burning of carbohydrate enables the complete oxidation of fats, and acidosis disappears. The normality of blood sugar relieves the depressing thirst and consequently there is a diminished intake and output of fluid. Since the tissue cells are properly nourished by the increased diet, there is no longer the constant calling for food, hence *hunger pain* of the severe diabetic is replaced by *normal appetite*. On the increased caloric intake, the patients *gain rapidly in strength and weight.* With the relief of the symptoms of his disease, and with the increased strength and vigor resulting from the increased diet, *the pessimistic, melancholy diabetic becomes optimistic and cheerful.*

Insulin is not a cure for diabetes; it is a treatment. It enables the diabetic to burn sufficient carbohydrates, so that proteins and fats may be added to the diet in sufficient quantities to provide energy for the economic burdens of life.

Stockholm 1921. P. A. Norstedt & Söner.

THE PHYSIOLOGY OF INSULIN AND ITS SOURCE IN THE ANIMAL BODY.

Nobel lecture delivered at Stockholm on May 26th 1925

by

J. J. R. MACLEOD.

The knowledge that the isles of Langerhans of the pancreas have the function of secreting into the blood a hormone which plays an essential rôle in the regulation of the metabolism of the carbohydrates, is the outcome of numerous investigations extending over many years, and to the development of this knowledge workers in various fields of medical science have contributed.

In 1889, when Minkowski and von Mering discovered that complete extirpation of the pancreas leads to fatal diabetes, practically nothing was known concerning the significance of the ductless glands, and few conceived that it would be possible to extract, from various of them, substances capable of replacing the lost function, when administered to animals from which some particular gland had been removed. Although, at this time, it was known that the thyroid gland is atrophied in myxoedema and in cretinism, it was not until 1892 that Murray discovered that administration of the gland removes the symptoms and it was only later that the doctrine of internal secretions, first enunciated by Claude Bernard in 1856 in connection with the production of sugar by the liver, came to take its place in physiological teaching. Minkowski in the complete account of his researches, published in 1893, considered the antidiabetic function of the pancreas to be dependent upon its acting as a ductless gland, and no doubt he had in mind that it performed this through an internal secretion, although the positive statement that such exists was first made by Lépine, who thought that it took the form of a glycolytic enzyme. But, so far, there was no hint as to the actual structure within the pancreas upon which the antidiabetic influence of the gland depends and it is primarily to the anato-

mists, Laguesse and Diamare, that we owe the hypothesis that this must be the collection of cells, named after their discoverer, the isles of Langerhans. By careful studies of the cytological characteristics of the cells of these islets, as distinguished from those of the much more numerous secreting acini among which they lie, and by painstaking examination of the anatomical relationships of the two kinds of cells in different classes of vertebrates, Laguesse and Diamare concluded that the islets must be responsible for the antidiabetic influence.

As this anatomical work was in progress, the potent action of extracts of the suprarenal gland on the blood pressure and other physiological functions was discovered, in 1894, by Oliver and Schäfer, thus adding strong support to the hypothesis that the ductless glands function by producing internal secretions. The hypothesis, that the islets of Langerhans of the pancreas must act in a similar manner, gained a firm hold among physiologists and clinical workers, with the result that many attempted to alleviate the symptoms of diabetes by administration of pancreas, or of extracts of the gland, to patients suffering from the disease. No success attended these attempts partly, we believe, because the antidiabetic principle was destroyed, either during the preparation of the extracts or by the action of the digestive juices, and partly, because of imperfect knowledge of the clinical course of the disease, particularly with regard to the relationship of diet to it. Notwithstanding the failure of these attempts, the hypothesis that the isles of Langerhans are the structures to which the pancreas owes its antidiabetic function was still maintained, and indeed strengthened, by the supporting evidence furnished by the graft experiments of Minkowski and Hédon. These workers showed that no diabetic symptoms supervene in dogs when a portion of the pancreas is transplanted into the wall of the abdomen prior to, or at the same time as, removal of the remainder of the gland, but immediately do so in full intensity when this graft is subsequently excised. Moreover, it was known that ligation of the ducts of the pancreas, or their injection by oil or paraffin, is not followed by diabetes. Since, in neither of these types of experiment, can any of the digestive secretion gain the intestine it was clear that the antidiabetic function of the pancreas must be independent of its digestive function. It may be well to point out also that the graft experiments once and for all disproved the view held by some (by Pflüger, for example), that damage to the nerve structures adjacent to the pancreas, or in the duodenal wall, is responsible for the diabetic symptoms.

A distinct step forward was taken in 1900 when Schulze and Ssobolew discovered that the degenerative changes which follow ligation of the ducts affect the cells of the acini much more markedly than those of the islets, and although among those who repeated these researches, there were some who failed to corroborate the findings, the conclusions of Schulze and Ssobolew were generally accepted. It was not long after this that the first, though unsuccessful, attempt was made to see whether an extract of the degenerated residue of duct ligated pancreas might not relieve the symptoms of diabetes. About this time also (1906) — as was revealed in 1922 by the opening of a sealed package deposited with the Société de Biologie — Gley had found similar extracts to diminish the symptoms in diabetic dogs and, in the same year, Miss De Witt had tried their effects on glycolysis.

The insular hypothesis of diabetes was meanwhile strongly supported by the careful histological studies of the pancreas of patients who had died from the disease (Opie) for, although it had been known, even prior to the experiments of Minkowski and von Mering, that the gland is often the seat of morbid change, it was not realised that the islets are the structures which are chiefly affected.

In 1903—04, Rennie, by anatomical studies in certain Teleostei, gave strong support to the view of Diamare, that the islet cells in these fishes exist as separate glands of relatively large size and more or less independent of the pancreatic acini. Both workers attempted to demonstrate an effect of extracts of these glands on sugar or starch solutions, but without success. They administered them by mouth to diabetic patients with no favourable results, although in one case, in which an extract was given subcutaneously, there was decided alleviation of the diabetic symptoms (Rennie and Fraser).

About this time the significance of internal secretions in the control of animal functions was clearly demonstrated by the discovery of secretin by Bayliss and Starling (1902), and the term »hormone» came into use to designate their active principles. Many believed that the antidiabetic function of the pancreas must depend on a hormone secreted by the isles of Langerhans, but neither the graft experiments already referred to, nor the transfusion experiments of Hédon — in which it was found that when the blood of a normal dog was transfused in a diabetic one the symptoms were alleviated — could prove the hypothesis. To do this it was necessary to show that extracts of the islets, or at least of the pancreas, are capable of

removing the symptoms of diabetes. In 1907 Zuelzer published results which must be considered, in the light of what we now know, as really demonstrating the presence of the antidiabetic hormone in alcoholic extracts of pancreas. But unfortunately, even although several diabetic patients were benefitted by administration of the extracts, the investigations were not sufficiently completed to convince others, and, apparently, Zuelzer himself was discouraged in continuing them because of toxic reactions in the treated patients.

To describe, even in mere outline, the further attempts to prepare active antidiabetic extracts of the pancreas would far exceed the limits of this essay. To Knowlton and Starling, Meltzer and Kleiner, E. L. Scott, Murlin and Cramer and to Clarke we owe much, for although none of these investigators succeeded in demonstrating beyond doubt that an extract having antidiabetic properties could be prepared from the pancreas, they all obtained results which were sufficiently positive to keep alive the hope that some day this would be possible. Special reference must also be made to the more recent work of Paulesco who prepared extracts having very decided effects on the sugar and the urea of the blood of diabetic animals.

Believing that the want of success to prepare extracts of uniform potency was due to the destruction of the antidiabetic hormone by the digestive enzymes also present in the gland, F. G. Banting suggested preparing them from duct-ligated pancreas, and with the aid of C. H. Best, and under my direction, he succeeded in 1922 in showing that such extracts reduced the hyperglycaemia and glycosuria in depancreatised dogs. The general symptoms of diabetes were also found to be alleviated and the duration of life of the depancreatised animal prolonged, by the repeated injection of alcoholic extracts of foetal, as well as of adult ox pancreas. Later it was shown, in collaboration with Collip, that other symptoms of diabetes, namely the ketonuria and the absence of glycogen from the liver, were favourably influenced by the extracts and, with Hepburn, that the respiratory quotient became raised. These results on depancreatised dogs showed beyond doubt that the antidiabetic hormone was present in potent form in the extracts and the time seemed ripe to investigate their action on the clinical forms of diabetes. This was done by Banting in a severe case under the care of W. R. Campbell, with the result that the hyperglycaemia and glycosuria were diminished. At the same time, however, it was found that it would be necessary to rid the extracts of irritating substances before the value

of their repeated injection in the treatment of diabetes in man could be adequately put to the test. This was accomplished by Collip, and the name insulin was decided upon for the purified extract. This name had previously been suggested by Sir E. Sharpey Schäfer (1916), who had been one of the first to support the hypothesis of the insular derivation of the antidiabetic hormone. I need nor here detail the rapid progress which it was now possible to make in studying the therapeutic value of insulin in the treatment of diabetes in man; for it is with experimental aspects of the subject that this essay is concerned.

The invariable lowering of the blood sugar which was observed to result from the administration of insulin in animals rendered diabetic by pancreatectomy, raised the question as to whether such would also occur in those forms of hyperglycaemia which can be induced by other experimental procedures, such as the injection of epinephrin, piqûre or asphyxia. As the first step in the investigation of this question, Collip injected insulin into normal rabbits and found the blood sugar to become lowered, thus furnishing a valuable method for testing the potency of various preparations and, therefore, for affording a basis for their physiological essay. At the same time it was found that neither piqûre, nor epinephrin, nor asphyxia caused any hyperglycaemia in rabbits in which, as a result of injection with insulin, the blood sugar was at a low level to start with.

Peculiar symptoms (convulsions and coma) were observed in many of the injected animals, and it was soon possible to show that these were related to the lowering of the blood sugar and that they usually supervened when this was about 0.045 per cent. Sometimes the animals recovered spontaneously from these symptoms, but more frequently the coma became so profound, with marked fall of body temperature, that death occurred. That the lowering of blood sugar is closely related to the occurrence of the symptoms, was proved by finding that the subcutaneous injection of a solution of glucose was followed, almost immediately, by complete recovery, even in cases in which death was imminent from deep coma. It has been found, in collaboration with Noble, that glucose is remarkably specific in this regard, the only other sugar which approaches it being mannose and, in certain animals, such as the mouse, maltose. Lævulose and galactose are decidedly inferior in their antidoting action, the pentoses are entirely inactive and none of the dissaccharides, other than maltose, has any effect. It is evident that this specificity in the action of glucose, in combating the hypoglycaemic symptoms, offers an opportunity to determine, not only

what related substances are readily converted into glucose in the animal body, but also what groupings in the glucose molecule itself are significant for the effects. By substituting various side chains in the molecule, as for example, by methyl groups, it has been found, in collaboration with Herring and Irvine, that none of these substitution products is effective, even such compounds as the mono-methyl glucosides being entirely inactive.

The fall in blood sugar is dependent upon increased diffusion of sugar into the tissues and not to its more rapid destruction in the blood itself. Thus, Eadie and I could detect no change in the rate of glycolysis by adding insulin to blood incubated under sterile conditions outside the body, or in blood withdrawn from animals injected some minutes before death with insulin. Hepburn and Latchford, on the other hand, demonstrated that the addition of insulin to the fluid perfused through the excised mammalian heart markedly increased the rate at which the percentage of sugar became diminished in it.

The striking relationship between the concentration of glucose in the blood and the normal functioning of the nervous system, which is revealed by these observations, had already been noted by Mann and Magath in their experiments on hepatectomized dogs. They observed that when the blood sugar fell to about 0.045 per cent characteristic symptoms supervened which could be antidoted by glucose, and to a less extent, by laevulose and mannose. We must conclude that when the tension of glucose in the tissue cells falls below a certain level (glucatonia), a condition of irritability becomes developed; but little is known as to what the underlying cause for this may be. Olmsted and Logan have advanced some evidence that it may depend on interference with the process of oxidation in the nerve cells, or that these are irritated by substances produced elsewhere in the body by faulty oxidation. More recent experiments by Argyll Campbell on the tension of oxygen in the tissues lend support to this view.

These observations emphasise the great importance of a certain tension of glucose within the tissue cells. They help us to understand why it is that the concentration of this sugar in the circulating fluids of animals of every order and species in which it has been determined, varies only within narrow limits, even after prolonged periods of starvation, or following muscular exercise.

We must imagine that it is by lowering the tension of glucose within the tissue cells that insulin primarily acts, so that the glucose of the blood plasma, with which the tissue glucose is in equilibrium, diffuses into the

Diabetes & Insulin

Nobel Lecture delivered at Stockholm, Sept. 15th 1923 by

Dr. Frederick G. Banting

cells to maintain the tension. With Eadie we have found that the free glucose extractable from the muscles by warm alcohol is reduced following the injecting of insulin, but we know nothing of the fate of the glucose which disappears. It is not converted into glycogen (McCormick, Noble and Macleod, Dudley and Marrian, Cori etc.) nor is it immediately oxidised, since the respiratory metabolism (intake O_2 and R. Q.) does not become increased at the time when the blood sugar is falling (Eadie, Dickson, Macleod and Pember; Trevan and Boock; Krogh; Boothby and Wilder etc.).

Although the intake of oxygen may become greater in certain animals such as dogs, cats and man when hypoglycaemic symptoms make their appearance this does not occur when sugar is also administered. In light of these results we have concluded that the glucose which disappears must become converted into some hitherto unidentified substance, but we have been unable to obtain any clue as to what this substance may be. Large amounts of it must be formed to account for the enormous quantities of glucose which may vanish from the blood, as when glucose is injected along with insulin. We have, for example, injected into rabbits, in the course of 8 hours, as much as 10 grams of glucose per kilo body weight along with insulin without finding, at the end, any increase in blood sugar, or in the free or the combined sugar of the muscles or liver. Burn and Dale have also shown that very large quantities of glucose can be injected along with insulin into eviscerated animals without increasing the percentage of the blood sugar. It is conceivable that between glucose and the material which is finally oxidised in the tissues there exists, not one, but a group of substances constantly changing from one into another in an equilibrated system, and that no one of them ever accumulates in sufficient quantity to make its identification possible by available chemical methods.

Be this as it may, it is significant that the percentage of inorganic phosphoric acid in the blood declines at the same rate as the sugar, although, in the recovery process, the phosphoric acid begins to rise decidedly before the sugar in animals injected with insulin. Accompanying this fall in the phosphates of the blood, those of the urine entirely disappear for several hours and then return to considerably above the normal level so that, in urine collected throughout the 24 hours, an excess is excreted.

[1] Practically all observers have confirmed the observation first made by Dickson and Pember that R. Q. rises somewhat in normal animals injected with insulin but the extent of this rise is not sufficient to indicate that increased combustion of glucose can be the significant cause for the rapid reduction in blood sugar.

as compared with the amount on days during which no insulin is given. (Winter and Smith, Allan and Sokhey, etc.). These facts would seem to indicate that in the process responsible for the disappearance of glucose in the tissues there is a stage when compounds of phosphoric acid with sugar or its immediate breakdown products are formed. One immediately thinks of the possibility that an increase in the amount of the substance, described by Embden and his school, in muscle, and named lactacidogen, might be responsible, but we have been unable to demonstrate that this is the case (Eadie, Macleod and Noble). At the present time we are entirely at a loss to account for the disappearing glucose. When this problem is solved it may be anticipated that a great advance will become possible in our knowledge of the intermediary metabolism of the carbohydrates.

Having outlined the known facts with regard to its physiological action, we may now turn to the interesting question of the source of insulin. The observations of Banting and Best, that simple extracts of the residue of pancreas remaining several weeks after the ducts are tied possess antidiabetic properties, does not necessarily prove that insulin is derived from the islets. As Bensley and others have shown there may still remain, at this period after duct-ligation, a considerable amount of more or less normal acinar tissue. Even were the gland allowed to degenerate for a sufficient time so that all acinar tissue had disappeared — which is considerably over a year in the rabbit — it would be difficult, in the event that extracts of the residue still contained insulin, to be certain that this insulin is not of the type which it is possible to extract from various materials, including even the tissues of depancreatised animals, as Best and Scott have shown.

Further investigation of the problem was therefore undertaken by continuing the work of Diamare and Rennie on certain of the Teleostei, such as Lophius and Myoxocephalus, in which the islet cells exist apart from the acinar tissue, as the so-called »principal islets«. Extracts were made by alcohol from these structures, as well as from the acinar tissue, and it was found that, whereas very large yields of insulin are readily obtainable from the islets, little or none at all can usually be prepared from the pancreas itself. Indeed, extracts of the latter sometimes cause the blood sugar of rabbits to become raised, instead of lowered. The lowering of the blood sugar by acinar extracts when it occurred, may have been due to the presence of a few scattered microscopic islets, such as have been observed by Slater Jackson to exist in the pancreatic bands of Myoxocephalus. That

the principal islet may have some acinar tissue associated with it does not detract from the value of the foregoing observations as evidence supporting the insular hypothesis, since it has been shown that extracts of the acinar tissue are comparatively impotent. In this connection it is of interest to note that insulin can be readily prepared from the pancreas of the Elasmobranchi (Raja and Squalus), which occurs as a compact gland with the islet tissue included in it, much in the same manner as in the mammalian pancreas.

But in view of the fact that insulin, or at least extracts capable of lowering the blood sugar in normal rabbits, can be prepared from other tissues than the pancreas or the principal islets, there still remains the possibility that it might be secreted internally from some of these. It is indeed possible that although this did not occur in the normal animal, in which a sufficient amount was coming from the pancreas, it might occur when the normal secretion was cut off, as in diabetes. By such a vicarious functioning of extra-pancreatic, potential sources of insulin, the tolerance of the diabetic organism for carbohydrate might become raised. It seemed important, therefore, to see whether diabetes would result from excision of the principal islets alone, an operation which is possible in Myoxocephalus, the two principal islets being readily removable without exposure of the fish to air for more than 15 minutes.

In a large number of fish in which this operation was performed it was found, in collaboration with McCormick, that the blood sugar became raised, often to ten times the normal value. At this high level it remained so long as the fish were kept alive — 11 days in one case, 5 to 10 days in others. The hyperglycaemia in itself was not sufficient to prove that the fish had become diabetic as a result of the isletectomy, for it was found, in other fish that were exposed to air for a period equal to that required for the operation (about 15 minutes), that the blood sugar rose, sometimes almost as much as in the operated ones. This asphyxial hyperglycaemia, however, was found to disappear within four days[1], nor while it lasted was it so pronounced as in the isletectomised fish. There is no doubt that removal of the islets in Myoxocephalus causes pronounced diabetes, as judged by the behaviour of the blood sugar, and, it is of interest to add that there was, on an average, considerably more fat and less glycogen present in the liver of the operated fish than in those of the controls. It remains to de-

[1] There was one fish in which the blood sugar remained at a high level even eight days after asphyxia.

termine whether, by giving insulin to the isletectomised fish, the blood sugar can be brought down to the normal level. So far we have been unable to demonstrate any very potent influence of insulin on the blood sugar of normal fish, although there is some indication that it can retard the development of asphyxial hyperglycaemia.

Taking all the evidence into consideration the conclusion seems justified that the only source from which physiologically effective insulin can be secreted within the animal body is the islet tissue.

And finally permit me to say something concerning the behaviour of depancreatised animals kept alive by means of insulin. By such studies it is possible that we may be able to determine whether the lost power to utilize carbohydrate can be reacquired in any measure, and also whether the secretion of pancreatic juice and of insulin include all the functions of the gland. With the collaboration of Frank N. Allan, I. L. Chaikoff, J. Markowitz and W. W. Simpson, several completely depancreatised dogs have now been kept alive, by daily injections of insulin, for many months, the operation on one of them, which is at present under observation, having been performed over eighteen months ago. But this result was not immediately achieved.

In the earlier observations it was observed that, notwithstanding the fact that the animals ate large amounts of meat, the body weight steadily fell, no doubt because of inadequate intestinal digestion and absorption. The addition of cane sugar, in amounts sufficient to cause a mild degree of glycosuria (50 to 100 grms daily), had the immediate effect of preventing the loss of body weight, and in most animals, of causing it to become increased, especially when large amounts were given. Four of these animals lived in excellent nutritive condition for periods varying between one and seven months, when each in turn developed symptoms of acute jaundice (bile pigment in urine, yellowing of sclera and skin) accompanied by rise in rectal temperature, anuria and progressive bodily weakness, ending fatally in from two to three days after the onset. The *post mortem* examination revealed, in each case, an extremely fatty liver with no significant pathological changes elsewhere in the body. Under the microscope it was difficult to see any liver cells that were not completely filled with fat, except for a few towards the centres of the lobules which were only moderately invaded. In some way or other, absence of the pancreas leads to a fatal breakdown of the hepatic function. Two possibilities may be considered: the one, that the pancreas secretes internally, besides insulin, some other hormone

which is necessary for the functional integrity of the liver, perhaps a hormone having to do with its action on fat metabolism; and the other, that in the absence of the pancreatic ferments the process of intestinal digestion becomes of such a (bacterial) type that substances having a toxic effect on the liver cells are absorbed into the portal blood. It was therefore decided to add raw ox pancreas (50 gm) to the daily diet, and it is as an outcome of this addition that the animals have thrived without showing any symptoms of hepatic breakdown. This favourable result may be dependent either on the restoration of the pancreatic enzymes, thus preventing the development of toxic substances, or because some hormone which withstands digestive action is absorbed from the ingested gland. We are at present observing the effect of adding trypsin to the food, instead of raw pancreas, but although the animal thus treated is in excellent nutritive condition we cannot as yet say whether it may not ultimately develop the hepatic symptoms. It may be added that the toxic theory is supported by the observation that in the absence of raw pancreas, or trypsin, not more than fifty percent of the ingested meat is assimilated, whereas over eighty per cent is assimilated when either of these is present.

The carbohydrate balance is being determined at intervals in several diabetic animals, in order to see whether any of the lost power to secrete insulin may be reacquired. This is done by determining the proportion of the ingested sugar which reappears in the urine daily while the animals are under the same dose of insulin, but so far no change has been detected. While it is certain that any considerable reacquirement of the power to secrete insulin would be revealed by this method, it is possible that a very scanty secretion might be masked on account of the relatively large amounts administered daily from without, for it has been shown, by Frank N. Allan, that the glucose equivalent of each unit of insulin is very much higher when the total number of units administered is small than when it is large. Another method for investigating this problem remains available, namely to observe whether the diabetic symptoms which supervene when insulin is discontinued are less severe after several months treatment than they are soon after the removal of the pancreas. Our attempts to make this observation have, so far, been frustrated by the very rapid downward progress of the animals after discontinuing insulin. Unless they are given large quantities of meat they die in a few days of symptoms not unlike those of diabetic coma.

It has been stated by Carlson and Drennan that the diabetic symptoms are very much less than usual when pancreatectomy is performed on preg-

nant animals near full time, and this they have attributed to the secretion of insulin from the foetal pancreas. We could obtain no evidence in support of this hypothesis in the present investigations. Thus, one of the depancreatised dogs gave birth to five pups without any change whatsoever in her sugar balance throughout the pregnancy, although, on the day after the pups were born, severe symptoms of hypoglycaemia developed, no doubt because of the removal of glucose from the body to form the lactose of the milk. There was therefore no evidence that the developing foetuses contributed any significant amount of insulin to the maternal organism. In the face of the relatively large amounts of insulin injected into the mother, however, it is possible, in view of Allan's results, that the small contribution from the foetuses could have no measurable influence on the maternal sugar balance.

I have attempted to review but a small part of the work relating to insulin and have only cursorily referred to the perplexing problem of the mechanism of its action in the animal body. Facts of importance in this regard come almost daily to light and it is to be anticipated that, as these accumulate, a great advance will become possible in our knowledge of the history of carbohydrates in the animal body.

Stockholm 1925. P. A. Norstedt & Söner