The Nobel Prize in Physiology or Medicine 1923

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Frederick G. Banting - Nobel Lecture

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Diabetes and Insulin

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, von Noorden, 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 gallstones. 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 sugar of the depancreatized dogs was 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 occurred 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.

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 hypo-glycaemic 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 1922, 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 with 70-95% 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 in 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.

Professor 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 pH 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 cc 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 g per 100 cc) 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 two 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 pH 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 pH 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 pH 5.0 and a white precipitate readily settles out. Tricresol is added to a concentration of 0.3% in order to assist in the iso-electric 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 iso-electric precipitation is carried out by adjusting the acidity to a pH of approximately 5.0. After standing overnight 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 hydrogenion concentration adjusted to pH 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 cc. 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 cc. 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 grams of carbohydrate to be utilized. In earlier and milder cases, as a rule, one unit has a greater effect, accounting for three to five grams 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. Campbell 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, Newburgh, and Holly that the body requires two-thirds of a gram of protein per kilogram of body weight per day (1 kilo=2.2 pounds) 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 58 per cent of the protein, 10 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 carbo-hydrates 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 was 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 symptoms 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 hypo-glycaemia 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 in coordination 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 occurred 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 hetaoxybutyric 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 grams 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 centimeters 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 or 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, he 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 grams 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 grams. 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 grams 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 acetone bodies made their appearance, and steadily increased. A distinct odour of acetone was at times distinguishable in 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 grams 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 it 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 g, fat 71 g, carbohydrate 20 g (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 g, fat 208 g, carbohydrate 97 g (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 g 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 45 to 105 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 134 pounds. Her present diet which is only approximate because she has dispensed with the weighing of food, is 125 g carbohydrate, 50 g protein, 50 g 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 g 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 hypergly-caemia are adequately controlled. To illustrate - Case 1, which required 6.9 units per 10 g carbohydrate in March, 1923, only required 2.6 units in January, 1924. Case 2, which required 7.8 units per 10 g in January, 1925, in June 1925 required only 2.8 units. Case 3, which required 6.5 units per 10 g 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 g 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 go units a year before, or, stated in another way, without insulin he could now handle 54 g 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 he 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:
(I) 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 130 children treated with insulin, 120 are still living, while of the 164 who did not receive insulin, there are 152 dead. Of the 120 still living, 40% have either not increased or have actually decreased their insulin. Dr. Joslin believes that if the 60% 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 maintained 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.

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