

# THE EFFECT PRODUCED ON DIABETES BY EXTRACTS OF PANCREAS

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IN the final report of his investigations on the effects of extirpation of the pancreas in 1893, Minkowski<sup>1</sup> does not speculate as to the manner of action of the gland. He considers the possibility that the pancreas acts in some way on the organs which use sugar so as to set free the "affinities" by which these attract the sugar molecules prior to metabolizing them. Although there is no mention of a possible internal secretion of the pancreas, the foregoing suggestion implies that Minkowski thought one might exist; but it is particularly to Hedon and Lepine that we owe the now popular hypothesis which definitely supposes this to be the case. Lepine thought that he could show that this hormone develops its function by causing glycolysis, and that the blood of a depan-created animal (and also of a diabetic patient) possesses a diminished capacity to break down sugar. This has been shown to be incorrect. Hedon sought to demonstrate the presence of the pancreatic hormone by transfusing blood from normal into depancreated animals. He found that this diminished the glycosuria of the latter, but subsequent investigations showed that the cause was not that greater consumption of dextrose had occurred, but that the excretory function of the kidney had become altered.

<sup>1</sup> Arch. f. exper. Path. u. Pharm., 1893, xxxi, 85.

In spite of the failure of these investigators to demonstrate that the diabetes which follows pancreatectomy is due to the removal of some pancreatic hormone that is essential for proper utilization of carbohydrate, the belief has steadily grown that such is really the case, and every now and then a paper appears in which attempts are described to demonstrate its presence. Many of these investigations have been of doubtful value because the sugar excretion by the urine has alone been studied, but more recently, since methods have become available for measurements of the percentage of sugar in the blood and the respiratory quotient, more definite results have been obtained. The problem has been to see whether changes are produced in the blood sugar or the respiratory quotient of diabetic animals when various forms of extracts of pancreas are administered.

In a general way it may be said that although results indicating a beneficial influence of such extracts have not infrequently been obtained in the experimental form of pancreatic diabetes, these have not been considered to be sufficiently constant or definite to warrant intensive study of the problem with a view to seeing whether favorable results might be obtained by similar treatment in human diabetes. The most noteworthy successes have undoubtedly been obtained by Murlin and Cramer, by Kleiner, and by E. L. Scott.

In none of the researches referred to, with the possible exception of that of Scott, is there any indication that sufficient attention was given to the possibility that the uncertainty of the action of the extracts might be due to the fact that these must usually contain powerful proteolytic enzymes which could digest or destroy any internal secretion also present. One of us (F. G. B.), impressed with this fact, undertook to reinvestigate the problem by using extracts in which the proteolytic enzymes were reduced to a minimum. It was decided to attempt this by taking advantage of the observation made first of all by D'Arnozan and Vaiclard, and afterwards confirmed by Sscobolew and by W. G. MacAllum, that ligation of the pancreatic ducts is not followed by diabetes, although apparently all of the acinar cells degenerate and disappear, leaving intact, however, some of the cells of the islets of Langerhans.

In two papers<sup>2</sup> Banting and Best have described in detail the effects which are produced on the metabolism of sugar in completely depancreated dogs by administration, subcutaneously and intravenously, of extracts of the residue of the degenerated gland made at ice-cold temperature with either isotonic saline or weak acid. Definite reduction of the percentage of blood sugar, lasting for several hours, accompanied by a marked decrease in the sugar excretion in the urine and by greatly augmented power of the animal to retain injected sugar, was observed. The extracts lost their potency by boiling and by digestion with pancreatic juice, but retained it for several weeks when kept in neutral reaction near the freezing-point. Similar extracts of other organs and tissues did not have these effects, except slightly in one case with extract of thyroid gland. There was no evidence that changes in the water content of the blood were responsible for the lowered percentage, of sugar.

It was repeatedly observed that the animals were greatly improved in their general condition by satisfactory injections of these extracts, and in one case (in which, however, whole gland extracts were used) life was prolonged to seventy days after the pancreatectomy, the average duration without treatment in our experience being within fourteen days and never greater than twenty-one. So far as could be judged by observation of this animal during life it was completely diabetic. It was killed by chloroform on the seventieth day and no trace of pancreas could be detected by naked-eye post-mortem examination. Serial sections of the duodenum, however, revealed a small isolated nodule of what appeared to be pancreatic tissue embedded in the mucosa. Further investigation of the longevity of diabetic dogs treated with extract is therefore necessary.

The success obtained by using extracts of degenerated pancreas and the uncertainty in the results of previous workers who used extracts of intact glands seem to support the hypothesis that the active principle, whatever it may be, is destroyed by the proteolytic (autolytic) enzymes also present in the latter. Encouragement was therefore given to seek for methods by which the destructive

<sup>2</sup> Journ. Lab. and Clin. Med., 1922, vii, pp. 251, 664.

action of these enzymes could be circumvented in extracts prepared from slaughter-house material. Since it is known that active trypsin is not present in extracts of the pancreas of the fetus of the ox until the fourth month, and since it is possible, as has been supposed by Carlson and Drennan, that the internal secretion which controls carbohydrate metabolism appears somewhat earlier, simple extracts were prepared from the pancreas taken from fetuses measuring about 40 cm. or less. These were found to be decidedly potent.

At the same time the trial was made to circumvent the destructive action of the proteolytic enzymes in adult gland extracts by using alcohol in place of weak saline solution as the extracting medium.<sup>2</sup> Working with small quantities of gland one of us (J. B. C.) succeeded by this method in preparing highly potent extracts that contained a low concentration of inorganic salts, no fats, only small amounts of protein, and were sterile bacteriologically. The clinical cases to be referred to later were treated with this extract which we propose to call "insulin." It is apparently quite harmless both to laboratory animals and to man when given in proper dosage, but in excessive amounts certain toxic symptoms supervene. It is partly because we desire to determine as accurately as possible the dosage necessary to cause these effects and partly because we wish to furnish the description of a method for the production of the extract in bulk and of constant potency that further details of this part of our work are at present withheld.

Some delay has been caused by great difficulties encountered in passing from small scale to large scale production, the opportunities for undertaking the latter being generously afforded by the Connaught antitoxin laboratories of the University of Toronto.. For two months, although apparently the same procedure was followed as in the small scale process, it has been found impossible to secure extracts on the large scale which are of adequate potency to justify their use in clinical practice.

Seven cases of diabetes mellitus have so far been treated with the extract under the personal supervision of two of us (W. R. C.

<sup>2</sup> An injection of an acholic extract had previously been made by one of us (F. G. B.) in a diabetic dog with the result that the blood-sugar was lowered by about 25 per cent.

and A. A. F.), and the preliminary results have been published elsewhere.<sup>3</sup> These have shown that subcutaneous injection of insulin causes the blood sugar to become markedly reduced even to the normal level, with disappearance of both sugar and ketone bodies from the urine. Evidence that these effects depend on an improved utilization of carbohydrate in the body was obtained! by observing the respiratory quotient. In two severe cases this value rose decidedly within a few hours after giving the extract, a result which -confirms that of similar observations on a diabetic patient and on depancreated dogs in the laboratory, the details of which will be published shortly. Not only were the objective symptoms of diabetes practically removed but a definite improvement was observed in the general condition of the patients, who also reported a subjective sense of well-being and of increased vigor for a period following each injection.

While it would be out of place here to give in detail an account of the numerous further observations which we have recently made on laboratory animals, there are certain of them which we believe should be alluded to partly because of their application in the control of the method of preparation of the extracts and partly because of the light which they throw on the mechanism of its action.

The following will be reported: 1. The effect of insulin on the blood sugar of normal rabbits and the occurrence of convulsions when this falls below a certain level.

2. The effect of insulin on rabbits rendered hyperglycemic in various ways.

3. The effect of insulin on the respiratory quotient of depan created dogs and of diabetic patients.

4. The percentage amounts of fat and glycogen in the liver and other organs of diabetic animals and the effect of administration of insulin on these.

1. THE EFFECT OF INSULIN ON THE BLOOD SUGAR OF NORMAL RABBITS AND THE OCCURRENCE OF CONVULSIONS WHEN THIS FALLS BELOW A CERTAIN LEVEL. In rabbits fed, as a rule, on oats, subcutaneous injection of from 2 to 10 cc of extract causes the

<sup>3</sup> Can. Med. Asso. Journal, March, 1922.

percentage of blood sugar to fall more or less rapidly, depending on the strength of the extract. This has made it possible for us to determine whether or not the various steps taken in the purification and concentration of the extracts were having the desired effect. When the extract is inactive the blood sugar of rabbits either remains constant or it may rise somewhat. As a result of 123 observations in which the blood-sugar curve fell it was decided to use as a standard of dosage the amount of extract necessary to lower the percentage of sugar by 50 per cent of the original in one hour, but this basis may have to be modified later. When the blood sugar reaches 0.045 per cent in a vast majority of cases typical symptoms make their appearance. The characteristic feature of these is that the rabbit lies on its side in a more or less unconscious state, often with the head retracted, and at the slightest stimulation (such, for example, as shaking of the floor) passes into violent convulsive movements which involve the entire body, causing the animal to throw itself about violently and roll over sideways, always in the same direction. In other cases the animal continues to lie on its side, but the extremities contract and relax as in running. This latter type of convulsive movement may apparently come on without exciting cause. The rectal temperature is usually about normal. These convulsive seizures recur at varying intervals, the animal becoming progressively more and more comatose in the intervals between them, and the breathing very shallow and rapid and frequently periodic in character. If left untreated the animal ultimately dies.

That these symptoms are related in some way to the lowering of the sugar is known by the fact that subcutaneous injection of 4 or 5 gm. of dextrose is followed by an immediate recovery, the animal sitting up and becoming perfectly normal in its attitude and movements. This recovery may persist or the convulsions may reappear after an hour or so, and when they do so, recovery may again be effected by injections of dextrose. The maximum of blood sugar at which convulsions were observed to occur was 0.045 to 0.047 per cent, with one exception at 0.067. The minimum at which no convulsions were observed was 0.037 per cent. The relationship of the convulsions to the degree of hypoglycemia

corresponds remarkably with the observations on Eck fistula dogs by Mann, and they undoubtedly indicate some important function between the sugar of the blood and the nutrition of the nerve centers.

2. THE EFFECT ON RABBITS RENDERED HYPERGLYCEMIC IN VARIOUS WAYS. For these experiments most of the rabbits besides being fed on oats were sometimes given cane sugar, so as to make sure that a large quantity of glycogen was present in the liver. They were then injected with active extract and shortly afterward were submitted to one or other of the methods usually employed for bringing about experimental hyperglycemia. These were piqure, subcutaneous injection of adrenalin, carbon monoxide and mechanical asphyxia. It was found that none of these methods produced hyperglycemia in the injected animals. Occasionally the blood sugar, previously lowered by the extract, might rise somewhat but never to anything like the extent ordinarily observed. Glycogen estimations of the liver after the experiment showed considerable quantities present. The importance of these results depends on the fact that they demonstrate the fundamental action of the extract on carbohydrate metabolism.

3. THE EFFECT OF INSULIN ON THE RESPIRATORY QUOTIENT OF DEPANCREATED DOGS AND ON A DIABETIC PATIENT. This part of the work is not as yet complete, but sufficient observations justify us in stating that administration of cane sugar or dextrose *plus* extract to completely depancreated dogs causes the respiratory quotient to rise much higher than it does when cane sugar alone is used. Thus in one experiment on a dog forty-eight hours after pancreatectomy ingestion of cane sugar caused the respiratory quotient to rise from 0.64 to 0.67; two days later a similar quantity of sugar *plus* extract caused it to rise to 0.85. The most striking effect, however, was obtained on a diabetic patient observed in the laboratory; cane sugar in this case caused no rise of the quotient, which stood at 0.75, whereas cane sugar *plus* 5 cc extract subcutaneously raised it to 0.94 after a delay of a little over two hours. This latent period in the effect of the extract seems to correspond to a certain stage in the lowering of the blood-sugar percentage, but it will take considerably more work before we can state this relationship precisely.

4. THE PERCENTAGE AMOUNTS OF FAT AND GLYCOGEN IN THE LIVER AND OTHER ORGANS OF DIABETIC ANIMALS AND THE EFFECT OF ADMINISTRATION OF INSULIN ON THESE. In confirmation of Minkowski, Cruickshank and others we have never found more than a trace of glycogen in the liver of completely depancreatised animals even after the ingestion of large quantities of sugar. The heart, on the other hand, contained usually about 1 per cent, which is considerably more than that of normal animals. Very different results were obtained when extract, as well as cane sugar, was given; thus in two cases 12.58 per cent and 11.4 per cent of glycogen were found in the liver, with 0.725 per cent and 0.570 per cent respectively in the heart. We have several other results of a similar nature. These striking differences indicate that one effect of the extract is to stimulate the glycogenetic function of the liver, and this, coupled with the fact that it also raises the respiratory quotient in an hour or two after its administration, suggests the possibility that carbohydrates can be utilized in the body only after they have gone through the glycogen stage. The fat has also been determined as fatty acid in the liver and blood and other organs with significant results; thus in the liver of diabetic animals without extract the percentage of total fatty acid in three cases was found to be 12.25 per cent, 14.1 per cent, and 9.9 per cent. In two animals treated with extract, on the other hand, the percentages were 7.42 and 2.19. The total fatty acid in the blood in two dogs without extract was 1.21 per cent and 1.12 per cent, whereas in two animals treated with extract it was 0.33 per cent and 0.51 per cent.

While these observations demonstrate conclusively that the pancreatic extracts, which we employed, contain some substances of great potency in controlling carbohydrate and fat metabolism in normal and diabetic animals as well as in patients suffering from diabetes mellitus, we cannot as yet state their exact value in clinical practice. We believe that dietetic treatment must still retain its place, but we expect that insulin will be of great assistance in carrying the patient over the various crises which are so frequently uncontrollable by other means.

The extract by which the foregoing observations have been



made was prepared on a small scale, and for the past three months, with the aid of the Connaught Laboratories, we have been endeavoring to work out in detail the preparation of large quantities. In this part of our work we have met with serious difficulties, but these we believe are now overcome, and we hope in the near future to be in a position to publish our method in detail.

#### DISCUSSION

DR. S. SOLIS-COHEN, Philadelphia, Pa.: Let me voice my admiration, and I believe the admiration of all present, for this extremely valuable and in many respects remarkable study. The idea of using the pancreas for the relief of diabetes has been in the minds of many ever since the observations of Lancereaux and those of Mittkowsky, but success has been reserved for Dr. MacLeod and his associates. I can appreciate their work the better for having been among those who have long vainly tried to find the road to the goal which they have reached. I suppose I have been worrying the life out of a certain physiological chemist who has been working upon this problem. About six months ago he thought he had succeeded; by in my last communication from him, about a month since, he said that he was not yet ready for clinical work.

I may, however, add this much to the discussion: At the Jewish Hospital of Philadelphia we have been using in my service for the last five years an imperfect preparation of the pancreatic enzyme, or hormone, or whatever it may be. Dr. David Kramer, my assistant there, is at present engaged in analyzing the results. We have had this experience repeatedly—unfortunately not in all cases, but in a sufficient number to encourage us to continue the use, even of this imperfect substance, and to make us devoutly wish for the better one that Dr. MacLeod has now found: Upon admission the patient is so dieted—only in very rare instances starved—that the urine becomes sugar-free. The diet is then gradually increased until the limit of carbohydrate tolerance is passed and sugar recurs in the urine. The pancreatic preparation is then administered, sometimes cutaneously, sometimes by mouth. The diet is maintained at its high level or even augmented, but the urine again becomes sugar-free, the blood sugar shows corresponding reduction and a higher limit of carbohydrate tolerance is definitely established. The fact that this higher level sometimes persists after withdrawal of the remedy is subject to different interpretations. It is a fact, but I will not now discuss its meaning.

If results of this character can be obtained— although not invariably—

with an inferior extract of the pancreas, we may surely have high hopes of the benefits to come from the active preparation of which we have heard today.

This study so careful and comprehensive, this work so thorough in its execution and so clear in its presentation, may justly be called epoch-making. I am glad that I have been privileged to hear the paper.

DR. R. T. WOODY ATT, Chicago, 111.: Having heard what Dr. MacLeod has had to say of this work in the meeting and out of it, I am convinced that he and his associates have actually had extracts containing the active principle of the internal secretion of the pancreas, and I think that this work marks the beginning of a new phase in the study and treatment of diabetes. It would be difficult to overestimate the ultimate significance of such a step. Heretofore we have managed diabetes by providing dietetic crutches. Out of this work there will develop in time a specific treatment. Apart from any immediate effect that it may have on the treatment of a single disease it must add significantly to our knowledge of metabolism and affect our thinking about the general problem of endocrine physiology. I move that the Association tender to Dr. MacLeod and his associates a rising vote expressing its appreciation of their achievement.

DR. F. M. ALLEN, Morristown, N. J.: Undoubtedly we are all agreed in congratulating Dr. MacLeod and his collaborators upon their almost miraculous achievement. Others have reduced glycosuria and hyper-glycemia with pancreatic extracts. I have done so myself; but the obvious reason why these experiments have proved nothing is found in the great toxicity of such extracts, so that the animals receiving them were injured instead of benefited and the fall of sugar could be attributed to intoxication. If, as seems to be the case, the Toronto workers have the internal secretion of the pancreas fairly free from the toxic material, they hold unquestionable priority for one of the greatest achievements of modern medicine, and no one has a right to divide the credit with them.

In the animal-experimentation which we have begun at Morristown within the past few months we have apparently demonstrated a reduction of diabetic glycosuria and hyperglycemia by pancreaticoduodenal serum in contrast to the negative results with serum from systemic blood. I had originally hoped to attack the problem in this indirect way, learning the solubility and precipitation reactions of the hormone in the relatively nontoxic pancreatic mixture. This investigation may perhaps still yield information of some value, even though it has been forestalled by the remarkably brilliant success of the Toronto group.

One word of warning may be proper concerning the practical possibilities: The discovery of the internal pancreatic secretion is not equivalent to a cure of diabetes. Unless means can be found for giving the extract

otherwise than by injection, and for securing more prolonged effects, the practical application of the discovery may be limited. We all hope for some practical benefits in supplementing the present diet restrictions and perhaps in acute emergencies such as coma, but the facts thus far known do not warrant any expectation of discarding diet treatment altogether.

DR. J. J. R. MACLEOD (closing): I wish to thank the Association very much in the name of my associates and myself for this very encouraging reception of our work. In regard to possible dangers in the use of the extract, I would point out that toxic effects are undoubtedly to be watched for. At the same time the patients treated by us were decidedly improved in their general condition, and there were no toxic symptoms. One of the patients (a young boy) had been lying in bed some time and had shown no interest in anything. Upon injection of the extract this condition changed wonderfully: The boy became much more interested in his environment and he showed a general improvement which was very striking to his relatives.

In dogs there is absolutely no doubt of the beneficial influence of the extract. The general well-being of the animal improves, the eye conditions become changed, the animal becomes anxious for his food and runs around the laboratory, where before he had no desire for food and never left his box.