BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 12 1940

A REVIEW OF THE CAUSES OF DIABETES MELLITUS*

BY

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LECTURE I

The complete removal of the pancreas of a dog by von Mering and Minkowski in 1889 showed that the pancreas was essential for the life of that animal, and it is now accepted that man also cannot live without it. Allen (1920) demonstrated that if one-sixth to one-tenth of the pancreas was left behind after operation the dog developed a mild diabetes. The fraction of the pancreas which is necessary to prevent diabetes occurring in man is unknown. The case of a woman was related (Graham, 1926) in which the body of the pancreas, together with a large portion of the head and most of the tail, was removed at operation. The head and body showed advanced fibrosis, but the tail was healthy. The fat-splitting and fat-absorption were normal, but the sugar-tolerance curve was abnormal three weeks after the operation. The patient reduced the carbohydrate of the diet, but when seen always passed sugar. The sugar-tolerance test gradually became more abnormal, but there were no symptoms of diabetes until sixteen years after operation. The slow decrease in sugar tolerance in this patient is similar to that described by Allen (1920) as occurring in his partially depancreatized dog, which was made to pass sugar by overfeeding with sugar, starch, or protein. The β cells lost their granules, developed vacuoles, and eventually broke up. This condition can be reversed by giving a low-calorie diet (Allen, 1920).

Changes in the β Cells

The observations on the partially depancreatized patient suggest that a gradual degeneration of the β cells occurred similar to that of Allen's dogs. Patients with mild diabetes who continue to pass sugar often have no symptoms for a long time, but eventually suffer from loss of weight and energy, and show other signs of the disease. This type of patient, like the partially depancreatized dog and patient, responds very well to insulin. If the carbohydrate of the diet is adequate and the blood sugar is completely controlled with insulin the patient may be able to give up insulin after a while.

The work on the pancreas suggests that the β cells are damaged, but we have no evidence as to the cause of the destruction, even in the acute cases where patients pass into coma within seven days of the onset of symptoms. The pathological changes in the pancreas have been studied by Opie (1910), Weichselbaum and Stangl (1902), Lane (1907), and Bensley (1911–12), and our knowledge is well summarized by Shields Warren (1938). The earlier workers described certain definite changes in the β cells, and their frequency is best demonstrated in Warren's table of his own cases.

He examined 534 cases, some of which showed more than one lesion. In 127 (or 23.7%) there were no pathological changes in the pancreas, and there was nothing to suggest that the patient had had diabetes; in 129 (24.3%) there was fibrosis of the β cells, but in only 12 (2.2%) were the changes well marked. Hyalinization of the island was present in 200 (37.4%) cases, and was well marked in 93 (17.3%). Hydropic degeneration was present in 22 (4.1%) cases, hypertrophy in 38 (7.1%), and minor changes in 18 (3.3%). The cases in which considerable damage was done were: 12 with fibrosis, 77 with hyalinization, and 24 with hydropic degeneration —a total of 113 (21%). These figures are very disturbing, especially when it is remembered that in 127 (23.7%) cases the pancreas appeared quite normal.

Warren points out that hyaline degeneration occurs chiefly in the elderly diabetics, often with a mild form of the disease, and that 50% of the patients known to have had diabetes for ten years showed this lesion. As fibrosis also tends to occur in the older people, both it and hyalinization may be characteristic of long-standing disease in the elderly. But both fibrosis and hyalinization occur in patients who have never had diabetes. Out of 200 cases 7.5% showed fibrosis and 2% hyalinization. The changes were as a rule slight, as only 1% showed marked fibrosis and hyalinization. Allen thought that hydropic degeneration was the characteristic lesion, as he found it in the partially depancreatized dogs who had been overfed with sugar and starch, and after long-continued protein overfeeding. Warren found it in only 5.5% of the diabetic patients, and it was present in 1% of the normal. It is, however, regarded as a change due to gross overwork or a wearing out of the cell, and it was expected that it would be a common lesion. It can be argued, however, that the β cells die off one by one, and that there are never many affected at any one time.

The Pituitary Gland and Sensitivity to Insulin

This deadlock was brought to an end by the work of Houssay and his collaborators in Buenos Aires. He and Magenta (1924) showed that if the anterior pituitary gland or hypophysis was entirely removed the animal became much more sensitive to insulin. Six years later Houssay and Biasotti (1930a, 1930b) found that they could produce diabetes in a toad by removing the pancreas, but that if the pituitary was first removed diabetes did not develop. It could, however, be produced at once if a graft of the pituitary was inserted. They repeated this work on a dog, allowing an interval for recovery (sixteen to sixty-one days) between the preliminary removal of the anterior lobe of the pituitary and the pancreatectomy. The diabetic condition produced was very mild, and only one dog out of eight needed any insulin, and then only for a period of nineteen days. These interesting observations suggested

* The Croonian Lectures delivered before the Royal College of Physicians of London on May 16 and 21, 1940.

that there was a very close relation between the anterior lobe of the pituitary and the islands of Langerhans, and that in the absence of the pituitary the amount of insulin necessary for the normal working of the body was much less than usual.

In 1932 three sets of workers—Evans and others (1932), Bauman and Marine (1932), and Houssay and others (1932)—all showed that when extracts of the anterior lobe were injected into dogs or rabbits the animals developed hyperglycaemia and glycosuria. The condition was, however, a transitory one, and ceased either quickly (Houssay and Marine) or slowly (Evans). Young (1937, 1938a, 1938b) confirmed these observations, and showed that if the dose of the extract was doubled the glycosuria returned, but again tended to disappear. If the dose was again doubled the glycosuria returned, and after one or more courses of treatment developed a diabetic condition which was permanent, although the injections were stopped. The diabetic condition differed in some important particulars from that which occurs after a pancreatectomy. The animals seemed well, tended to gain weight, and had more glycogen in their liver than depancreatized dogs. Although the glycosuria could be controlled with insulin the animals kept well without it for long periods if they were given enough food to eat. They responded to the hypoglycaemic action of insulin, but nothing like so well as a depancreatized dog. When the dogs either died or were killed the pituitary, adrenal, and thyroid glands were all normal, but the pancreas showed definite changes in the islands. These, however, were not constant, since either many of the islands might be completely replaced by hyaline material or the β cells of the islands might only be reduced in number, leaving the α cells unaffected (Richardson and Young, 1938; Richardson, 1940). Although hydropic degeneration occurs they suggested that it was seen only when the pancreas was subjected to a great rush of work. The pancreas of one dog appeared normal except for a decrease in the number of cytoplasmic granules in the β cells. The changes in the pancreas are almost as varied as those which are seen in the human pancreas. Yet it seems certain that the β cells are damaged by the pituitary extracts and that the diabetes arises as a result of the destruction caused by them, since the amount of insulin produced by the pancreas is much decreased. Thus Campbell and Best (1938) removed the pancreas from a dog which had been rendered diabetic with pituitary extracts. The insulin required to keep the animal well on a diet containing 85.6 grammes of glucose was 1.69 units per kg., or 16 units, while 11.2 grammes of glucose was excreted. After the animal was depancreatized the same amount of insulin was necessary, but the sugar excretion was rather higher at 18 grammes. This suggested that the amount of insulin secreted was very small, and on assay the pancreas yielded only 2.5 units instead of 80. This has been confirmed by Marks and Young (1939b).

So far it has been found impossible to make a rat diabetic with pituitary extracts, and the amount of insulin secreted has increased by 130% to 250% (Marks and Young, 1939a). Young failed to make one dog diabetic, and in this case the islands of Langerhans were bigger and much more numerous than usual.

Marks and Young (1940) have shown that an insulin-increasing or pancreatotrophic substance is present in the hypophysis, but that it is not identical with either the diabetogenic or the growth substance. An extract which increases the islets in the rat may cause diabetes in the dog, or it may be innocuous if the diabetogenic substance is not present. The discovery of a pancreatotrophic substance raises the hope that it may be active in human beings and so stimulate the growth of the β cells. If this hope is realized it may be possible to cure patients who make only a small amount of insulin.

The destruction of the β cells in any individual case may be due either to a susceptibility of these cells in patients who have a family or racial history of the disease or to a great excess of the diabetogenic substance. Further, the lesions in the dog's pancreas resemble those found in 50% of Warren's patients who had had diabetes for more than ten years. This type of patient is as a rule very easily controlled by insulin and is not liable to suffer from sudden attacks of hypoglycaemia. The insulin dosage of my partially depancreatized woman has been very constant in the last two years and lends support to the view that in this type of diabetes the patient has an extensive lesion of the pancreas and is making too little insulin.

Substances inhibiting Insulin Action

The intensive study of the various substances extracted from the hypophysis has shown that another substance has an effect on insulin: Young (1938a, 1938b) has demonstrated that a substance separated from prolactin, which is concerned with the crop function of birds and milk production of mammals, inhibits the action of insulin. It seems possible that this glycotrophic substance has an important role. It is apparently similar to that prepared by Collip (1938), which also inhibited insulin. Pituitrin, which is extracted from the posterior lobe, also has the power of inhibiting the action of insulin. This was first shown by Burn (1923) working with rabbits, and by Lawrence and Hewlett (1925) on human beings, with and without diabetes. Pituitrin is of great value in preventing hypoglycaemia and also in raising the blood sugar after hypoglycaemia. The property of antagonizing insulin is shared by the two substances present in pituitrin, but the anti-insulin effect of vasopressin is about twice as great as that of oxytocin (Gurd, 1934). There are thus at least two substances—the glycotrophic factor and pituitrin—which inhibit the action of insulin and may well be responsible for the insulin-resistant cases, and there should therefore be at least two types of diabetes.

It is well recognized that it is quite impossible to say, by estimating the blood sugar, how much insulin a patient will require to lower the blood sugar to normal. The dose may be a small one—10 to 20 units—or it may be 100 to 200 units or more before the diabetic condition is controlled. The largest amount recorded is 3,240 units a day, and in spite of this heroic dose no less than 82 grammes of sugar was excreted. Some months later the dose had decreased to a mere 400 units (Wiener, 1938). Many of these insulin-resistant cases are fairly stable and do not suffer from severe attacks of hypoglycaemia, except when they begin to improve. Others, however, are very unstable and may suffer severely from attacks of hypoglycaemia.

Types of Diabetic Patient

Himsworth (1936) and Himsworth and Kerr (1939), as a result of work on sugar-tolerance and insulin-depression curves, concluded that diabetic patients could be divided into two main types—the insulin-sensitive and the insulin-insensitive or resistant. Himsworth examined 36 cases by a most time-consuming method: 14 were sensitive and 22 insensitive. He thought that the two types were quite distinct. De Wesselow and Griffiths (1938) repeated this work, and while they agreed that there were two types of disease they did not think that the types were pure, but that some cases were of a mixed type. Their experiments were criticized by Himsworth and Kerr (1939) on the ground that they did not estimate the blood sugar at such frequent intervals as the latter workers, who think the difference between the conclusions can thus be explained.

I find it difficult to believe that the two types are so clear-cut for the following reasons: (1) It does not agree with my clinical experience, for I have seen patients with an acute attack of diabetes whose condition often improved so much that the insulin was either given up or was continued in a much reduced dosage. After a variable period of time the condition is difficult to control, and some of these patients become definitely insulin-resistant. (2) Although the conditions of the test were very carefully chosen I think an important control was omitted. The patient was given nothing to eat after 6.30 p.m., and the test began at 10 a.m., some two to three hours after the usual time of breakfast. One of the difficulties of controlling diabetics is the tendency for the blood sugar to begin to rise before the morning injection, and in some cases this takes place one to two hours before the injection. In Himsworth's cases the effect of the evening insulin may have worn off by 10 a.m. and the blood sugar may have been about to rise in his insulin-insensitive cases. If this were the case it would explain why the blood sugar rose so rapidly after sugar and could not be controlled by the insulin. If a control without sugar or insulin had been carried out two days before the first test the experiment would then have been above suspicion.

The clinical evidence for the insulin-resistant type is strengthened by the work on the glycotrophic substance. It would be natural to expect that this substance would be present in the blood. Boller, Uiberrak, and Falta (1934) and de Wesselow and Griffiths (1936) reported experiments which suggested that the blood of insulin-insensitive patients contained a substance which inhibited the action of insulin, but Himsworth and Kerr (1939) were unable to confirm this. It is possible that this is due to the small amount which is present in the blood at any one time; more might be excreted in the urine and might be more easily isolated, but so far this has not been reported.

The evidence, therefore, is in favour of two main causes of diabetes: in one the β cells are damaged and produce very little insulin; and in the other the β cells are normal, but, owing to the presence of an interfering substance, fail to produce enough insulin, although they may produce a great deal.

Insulin Dosage

These two causes will explain the great majority of the cases of diabetes, but certain difficulties remain to be explained. The first of these is the respective size of the morning and evening doses of insulin. I would have expected that the morning dose of insulin would be greater than the evening dose, because it has to look after the carbohydrate of three meals—some 150 grammes of carbohydrate—and the evening insulin only one meal—C. 50 grammes, if 200 grammes of carbohydrate is eaten; but this is not the case. I have analysed the figures for 243 patients who have been attending regularly in my follow-up department and whose blood sugar is under reasonable control. In 86 (35.4%) the dose was exactly the same. In 112 (46.2%) the morning dose was 5 units greater than the evening dose. In 23 (9.47%) the difference was between 5 and 10 units, while in 7 (2.48%) it was over 10 units. Further, the evening dose was greater by 1 to 5 units in 11 (4.53%) cases, in 3 by between 5 and 10 units, and in 1 by over 10 units. The figures for the protamine-zinc-insulin are also instructive. In 24 cases protamine-zinc-insulin by itself controlled the diabetic condition, but in 13 the dose of soluble insulin was equal to and in 32 was greater than that of protamine-zinc-insulin, and in 11 the dose of protamine-zinc-insulin was greater than that of soluble insulin. The finding that the morning dose is not always greater than the evening dose can be explained by saying that the body's need of insulin is considerable during the night in some cases. It is easy to understand this being so if, for instance, the patient has an active tuberculosis with fever; but that is not the case with my patients.

It is equally difficult to explain the condition in which the blood sugar swings quickly from a hyperglycaemia of, say, 300 mg. to a hypoglycaemia of 40 to 50 mg. some four to six hours later, and back to a hyperglycaemia of 250 to 300 mg. in another six hours. This may take place with quite small doses of insulin—10 to 20 units—or with big doses of 100 units or more. This type of patient needs either three or four doses of insulin to control the diabetic condition, so protamine insulin was specially introduced by Hagedorn to avoid the inconvenience of the multiple injections. I have used this insulin in combination with soluble insulin on 48 patients who attend at hospital regularly and whose blood sugar at midday is fairly well controlled. In 19 cases a mixture of the two kinds of insulin is needed twice a day, because the blood sugar tends to rise rapidly both at 8 a.m. and at 7 p.m. In 12 a mixture of the two insulins was required in the morning, but the soluble insulin was sufficient in the evening, as the blood sugar did not rise before 8 a.m. In 1 a mixture was necessary in the morning, but in the evening protamine insulin was given alone, since the patient was liable to have hypoglycaemia before midnight if any soluble insulin was given. In 5 cases it was unnecessary to give any protamine insulin in the morning, but at night either a mixture or protamine alone was used. In 11 cases the soluble insulin, with its quick action, could not be given in the morning because of the risk of hypoglycaemia at midday. In 6 of these cases the soluble insulin was sufficient at night, but in 5 protamine insulin was necessary. Finally, the length of time which the different kinds of insulin take to cause hypoglycaemia varies greatly. In the majority of cases hypoglycaemia occurs three to four hours after the injection of soluble insulin, but in a few it may not take place until nine or ten hours after the morning or evening injection. The effects of protamine insulin also vary very much. In some cases its action is as quick as that of soluble insulin, while in others it is very slow. One patient has a dose of protamine insulin in the morning, and is liable to have hypoglycaemia some eighteen to twenty-four hours afterwards.

Variations in Response to Insulin

Again, one of the difficulties of looking after a bad diabetic is the varying response to insulin. This became especially noticeable when protamine insulin and protamine-zinc-insulin were used to control the difficult cases. Thus, on one day a patient may pass no sugar at all, the next day he may pass a good deal of sugar, and the following day he may have an attack of hypoglycaemia, though the dose of insulin is not changed. At first I ascribed this to the irregularity of the absorption of the insulin, but I have since observed it when soluble insulin is used, and think that it is due to some change in the patient's condition.

I find it very hard to attribute these fluctuations and variations of response to the damage of the β cells which has reduced the yield of insulin. It is impossible to explain them by the action of the glycotrophic substance if the same amount of this is present each day. If, however, the supply of the glycotrophic substance varies from day to day and from hour to hour it would explain why the response of the patient differed so much. It would

also explain the curious variation in the amount of protamine insulin and soluble insulin to which I have referred, the rapid swing from hyperglycaemia to hypoglycaemia and back again to hyperglycaemia, and the difference in the size of the two doses of insulin. This hypothesis of the varying activity on the part of the hypophysis is a pleasing one, as it will explain so many difficulties; but there is at present no direct evidence for it. If the amount of the glycotrophic substance and of insulin in the blood and urine can be estimated it should be possible to prove or disprove this hypothesis. The only direct evidence for the varying activity of the hypophysis is that provided by Dr. Leonard Mark (1912) in his observations on his own case. He described what he called an acromegalic state. This might come on at any time of the day, but was most common during the latter part of the morning. Its duration was very variable: it might last only about half an hour or it might be present all day. When he was in the acromegalic state his general appearance altered; his hands and feet were bigger, cold, and moist; his eyes were more sunken, and his tears flowed abundantly—so much so that the silk lapel of his frock-coat rotted. I have not found any other reference to this condition, as Harvey Cushing (1932) and Atkinson (1932) do not mention it. If, however, Leonard Mark's observations are correct the acromegalic state can only be due to a variation in the activity of the hypophysis, and if this takes place in acromegaly it may also occur in the secretion of the glycotrophic factor, and so explain many of our present difficulties.

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Y. M. Vesval (*Thèse de Paris*, 1940, No. 182), who has collected 57 cases of agranulocytosis in children aged from 3 months to 14 years, including a personal case in a boy aged 3½ years, states that twenty-eight were boys and twenty-nine girls. The prognosis is very grave. As in the adult the aetiology is very obscure; the occurrence of the disease may sometimes be explained by a toxic or more frequently an infective factor perhaps associated with a special predisposition of the bone marrow. Agranulocytosis in the child very frequently masks an acute leukaemia, and an early and correct diagnosis can only be obtained by a study of the myelogram.

CHANGES OCCURRING IN BLOOD STORED IN DIFFERENT PRESERVATIVES

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The object of the present investigation was to study the changes that occur in certain elements of the blood stored in different preservatives.

Methods of Investigation

The following characteristics were initially chosen for analysis: (i) total red cell count and red cell fragility in hypertonic saline; (ii) total white cell count and differential count; (iii) platelet count; (iv) sedimentation rate; (v) coagulation time. The red cells were counted in a Bürker counting chamber, using a 0.5% solution of gentian violet in normal saline as diluting fluid (Price-Jones, Vaughan, and Goddard, 1935), and the fragility was determined by the method of Dacie and Vaughan (1938). The platelets were counted by Lempert's modification of Kristenson's technique (Lempert, 1935); the sedimentation rate was done by Wintrobe's method (Wintrobe and Landsberg, 1935); and the coagulation time by a modification of Lee and White's (1913) technique, using a dry instead of a wet tube.

After a few observations it was found that the most striking effect of changing the preservative was upon the red cells. In subsequent experiments, therefore, the red cells only were studied. The blood was withdrawn by gravity (Vaughan, 1939). All examinations were made on small samples taken from the full bottle of blood at the time of examination. In many instances daily estimations were made, though for clarity only the weekly results are recorded in the tables. The solutions were:

1. Saline citrate solution (1.05% sodium citrate in 0.85% sodium citrate).

2. The same saline citrate with the addition of 0.3% glucose (giving a final concentration of 0.1%).

3 and 4. Both the above solutions fully oxygenated.

5. The saline citrate solution with 3% glucose (giving a final concentration of 1%).

6. The saline citrate solution with 6% glucose (giving a final concentration of 2%).

7. Caramelized 0.3% glucose saline citrate.

In all cases 2 parts of blood were added to 1 part diluent.

These solutions were chosen because, with the exception of No. 7, their use had been advocated by other workers.

If glucose is autoclaved with sodium citrate caramelization occurs. Although glucose can be added after the sodium citrate solution has been autoclaved, it is a rather cumbersome procedure. It therefore seemed worth while to determine whether the caramelized solution had the same preservative effect. Caramelization decreases the amount of reducing substance present.

Results

Sedimentation Rate.—As other observers have pointed out (Filatov, 1937; Macdonald and Stephen, 1939; Fahraeus, 1939), the sedimentation rate is retarded in stored blood. This appeared to be slightly less definite in solutions containing glucose, but the figures available allow of no definite conclusions on this point.