INSULIN DEFICIENCY AND DIABETES

By

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THEORETICAL PART

Diabetes is commonly considered to the result of an absolute or relative insulin deficiency or hypo-insulinism, but whether this is a true interpretation is not clear.

Given a normal requirement of insulin, the inability of the B-cells to secrete sufficient insulin, gives rise to an absolute deficiency, while increased requirement of insulin, may lead to an inability to produce sufficient extra insulin, which is called a relative deficiency. Such an increased demand on the insulin secreting cells, with the possibility of overtaxing the B-cells, may be experimentally induced, e.g. when large amounts of glucose are infused into normal animals. This is a pure form of relative insulin deficiency. If in the human, obesity and overeating results in diabetes, the same mechanism may be involved. The possibility remains, however, that in some of these clinical cases, an absolute insulin deficiency develops, caused by overstraining and consequent degeneration of the B-cells, so that permanent diabetes may remain after the cessation of hyperphagia and obesity. This can also occur in animals loaded with glucose.

An increased demand on the insulin secreting cells may also be caused by the preponderance of insulin antagonists: e.g. by the hypophysis and its subordinate organs (adrenals, thyroid) and presumably glucagon. This may lead to an absolute deficiency, providing the B-cells are damaged functionally.

It is evident, that we know the cause of experimental insulin deficiency, but in most instances of human diabetes, we can only guess about the etiology.
of the case. There can be either one process, which leads to a functional inferiority of the islet cells, as well as to preponderance of insulin antagonists or other factors may be involved. For the sake of simplicity we assume, that one process, which we call »the basic process«, causes an absolute or relative insulin deficiency. We do not know, however, whether the whole clinical picture of diabetes is caused by insulin deficiency: one may consider the possibility, that certain symptoms are caused by the same basic process which leads to insulin deficiency. In other words, there need not be a causal relationship between all these symptoms and the insulin deficiency, both aspects being independent consequences of the same cause.

Of course we realize, that insulin deficiency causes disturbances in various kinds of metabolism and that several of these disturbances manifest themselves in clinical symptoms, but in the picture of diabetes, there is a group of fairly well defined clinical manifestations, which may not be due to insulin deficiency, e. g. retinopathy, premature atherosclerosis, the Kimmelstiel-Wilson syndrome, neuropathy and other effects of so called long term diabetes, with the exception of cataract.

When considering the question of late complications, it is important to be clear about their origin, since, if insulin deficiency is not the cause, correction of insulin deficiency might prove to be inadequate in the prevention of these complications.

This problem, however, is even more important from a theoretical point of view, since it casts doubt on the reliability of the generally accepted conception that disturbances of carbohydrate metabolism, leading to hyperglycemia, are causative factors in the development of diabetic complications.

Exception should be made in the case of cataract, since it has been shown to develop as a rule in alloxan treated animals, and in that case can be prevented by insulin treatment (Cohen et al., 1953). Diabetic cataract is an effect of insulin deficiency.

Research in the field of premature atherosclerosis of diabetics is more involved with cholesterol metabolism and lipo-proteins than with carbohydrate metabolism, and hence not, as believed, essentially due to hyperglycemia.

It is not clear, why disturbances in carbohydrate metabolism have gained such a predominant position in current considerations. Perhaps this is due to the fact, that both hyperglycemia and glucosuria are so easy to determine. Moreover it is not justifiable to blame any special aspect of insulin deficiency such as hyperglycemia, as the only cause in the development of diabetic complications, since it might be questionable whether insulin deficiency as a whole is responsible for these complications.

It is remarkable that this point of view seems to have been neglected in the study of diabetes!

One argument, that argues against insulin deficiency as the cause of diabetic
complications, is found in the study of Duff & McMillan (1949) who observed that, in spite of a high blood cholesterol level, alloxan-diabetic rabbits are less sensitive to atherosclerosis after cholesterol feeding than their normal controls. This work has been confirmed by McGill et al. (1949).

However, the finding of Foglia et al. (1950) that in depancreatized rats a picture similar to the Kimmelstiel-Wilson syndrome in man develops, may be an argument for those, who advocate the insulin deficiency theory as the cause of late diabetic complications. Reviews by Lukens & Dohan (1946) and Lukens (1950) in the literature on the relationship between experimental insulin deficiency and complications, do not allow of a definite conclusion, and in spite of an intensive search in the literature, we could not find any further evidence bearing on this problem.

Thus, it may be stated, that there is but little experimental evidence for the assumption, that insulin deficiency is responsible for all the diabetic complications except cataract.

In the absence of convincing experimental evidence, clinical experience may give us the answer to the question, namely whether unsatisfactory control, i.e. incomplete correction of insulin deficiency, might be responsible for the late diabetic complications. Bad control may be due to several reasons. One reason may be the carelessness of the patient or neglect in following the physicians prescriptions. Although we do not underestimate the importance of this lack of cooperation on the part of the patient, we can not attribute a subsequent failure of our therapy, i.e. the development of complications entirely to the faults and errors of the diabetic subject himself.

Another cause of bad control may be the impossibility of replacing natural insulin secretion by the injection of insulin. This may be due to unsatisfactory types of available insulins, but in many cases, the failure to achieve satisfactory control lies in the character of the diabetes. If good or bad control is due to the nature of the particular types of diabetes, it is not remarkable that severe diabetes (with bad control) should give complications and mild diabetes (with good control) should not. As pointed out above, it is not certain whether insulin deficiency is the cause of the complications and thus it is possible, that both insulin deficiency and late diabetic complications have a common cause. When bad control and complications are found in the same case, it is not necessarily correct to ascribe the diabetic complications to bad control. They might both be effects of the same cause and show no cause-effect relationship, but appear to be parallel manifestations.

There is some evidence, that not insulin deficiency per se, but a preponderance of insulin antagonists originating from the hypophysis, adrenals, thyroid are responsible for the occurrence of late diabetic complications.

In the first place, there is the unique observation of Poulsen (1953), in which a diabetic woman with a retinopathy, became pregnant. After delivery a
Sheehan syndrome developed, with the result that, with the depression of the pituitary function, the retinopathy disappeared.

Becker et al. (1954) reviews the probability of increased function of the adrenal cortex as being the cause of diabetic complications. Though it is too early to make any definite statements about his hypothesis, it is important, that this point of view should be considered.

In cases in which pituitary (adrenal, thyroid) preponderance is considered to play a rôle in the etiology of insulin deficiency, it is known that this insulin deficiency can be improved by hypophysectomy (Houssay effect), adrenalectomy or thyroidectomy. These methods have also been advocated for the treatment of diabetic complications (Lauf et al., 1953, Kinsell et al., 1954, Wortham et al., 1954, Martin et al., 1954), but are only justified in human subjects, when a direct effect on the complications is assumed to exist. When a possible beneficial influence of hypophysectomy, adrenalectomy or thyroidectomy is considered as a means of improving the balance between insulin and its antagonists, i.e. a correction of insulin deficiency, it might be advisable to use the less dangerous procedure of administering insulin.

Doubt is cast on the assumption, that insulin deficiency is the cause of diabetic complications, by a remark of Wilder (1946), who pointed out, that our correction of insulin deficiency has resulted in a marked fall in the incidence of coma, i.e. in the incidence of dangerous dehydration and ketosis, but the late diabetic complications do not appear to be influenced by our insulin therapy.

Summarizing it may be stated, that there is no experimental or clinical evidence, which supports the commonly accepted belief, that insulin deficiency is the cause of late diabetic complications. Until this has been proved, it is more correct to speak of «experimental insulin deficiency» and not to use the expression «experimental diabetes» since it is possible that insulin deficiency is only a part of the clinical syndrome: diabetes.

The lack of experimental data on this essential and fundamental problem of diabetes, induced us to attempt to find out whether it is possible to demonstrate the occurrence of late diabetic complications in the course of experimental insulin deficiency.

**E X P E R I M E N T A L P A R T**

Male albino rats weighing between 125 and 150 gm. were injected subcutaneously with a single dose of 75 mg./kg. alloxan in aqueous solution. The animals were placed in metabolism cages. The glucose content of the urine was first estimated qualitatively with Benedict's reagent and, in cases of positive reduction, quantitatively by polarimetry. All animals showed a glucosuria during the first week after alloxan treatment. The animals, in which this was not permanent, were used as controls: during the remainder of the experimental period they never again had a positive glucose reaction in their urine. The other animals had a permanent glucosuria.
During the first week, the urine was collected continuously and analyzed in 3-day portions. Later on the animals were placed for three days each week in metabolism cages and the urine voided during this period, examined. The animals were weighed every week. They had the usual rat diet (Bertels, Amsterdam) and tap water, ad libitum, at their disposal.

The experiment lasted for 323 days. The rats were then killed with chloroform and a number of organs removed for microscopic investigation, as described in a subsequent section.

Results:
Only those animals, which survived the entire experimental period, are described. Thus we had at our disposal 3 controls (Nos. 8178, 8181 and 8185) and 5 alloxan insulin deficient rats (Nos. 8179, 8184, 8186, 8189 and 8193). All these animals remained healthy, except No. 8189, which became cachectic during the last month.

The mean body weights are given in Fig. 1. It is seen from the graph, that the glucosuric animals, during the first 2 months, had a smaller increase in body weight than the controls. In the subsequent period, the growth curves are fairly

![Graph showing body weight changes over time.](image-url)

**Fig. 1.**
Average body weight of 3 controls and 5 alloxan-treated rats.

**Table 1.**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Average glucosuria (entire experimental period)</th>
<th>Range</th>
<th>Average daily urine production ml.</th>
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<tbody>
<tr>
<td>8179</td>
<td>9.7 %/o</td>
<td>6.0–15.4</td>
<td>47</td>
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<tr>
<td>8184</td>
<td>9.5 %/o</td>
<td>7.0–13.4</td>
<td>48</td>
</tr>
<tr>
<td>8186</td>
<td>9.5 %/o</td>
<td>6.0–13.6</td>
<td>43</td>
</tr>
<tr>
<td>8189</td>
<td>7.1 %/o</td>
<td>4.2–10.0</td>
<td>68</td>
</tr>
<tr>
<td>8193</td>
<td>10.3 %/o</td>
<td>7.0–15.4</td>
<td>36</td>
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</table>
parallel. The decrease in mean body weight of the insulin deficient animals at the end of the experimental period is caused by the fact, that the rat, mentioned previously (No. 8189) lost considerable weight – from 169 to 130 gm. in a month.

The degree of glucosuria is shown in Table 1. All insulin deficient animals had a continuous, rather severe glucosuria and a marked polyuria, as compared with the controls – No. 8178, 8181 and 8185, which had an average daily urine production of 22.5 and 7 ml. respectively and no glucosuria.

Pathological findings:
Methods and technique.
Immediately after death, pieces of the liver, kidney, abdominal aorta and femoral artery were removed. Fixation was in neutral formol, 4%, Bouin's fluid and alc. absol. These specimen were inbedded in paraffin and sectioned at 4–5 µ.
After mounting they were stained with haematoxylin-eosin, v. Gieson, Masson's trichrome, periodic acid Schiff's reagent (PAS) and Best's stain for glycogen.
Frozen sections of formalin fixed tissue were prepared from the arteries, liver and kidney and stained with oil-red 0 (Sudan IV).
The basal membranes of the glomerular tufts were studied according to the boiling fixation method (Lu, 1923).

Results:
Liver: no apparent abnormalities. In 4 rats (2 insulin deficient, 2 controls) there were small focal necrotic areas, possibly as a result of alloxan intoxication (Cruickshank, 1954). No abnormal steatosis or glycogen accumulation.
Arteries: in both groups of rats the elastic and muscular arteries appeared to be normal. No lipids were present.
Kidneys: the alloxan insulin deficient rats showed a marked swelling of some loops as compared with the control rats (Fig. 2). Unlike Foglia (1950) we could not find any signs of intercapillary glomerulosclerosis. The cachectic rat (No. 8189) had a purulent pyelonephritis.
Glycogen in the tubular epithelium was found only in the insulin deficient rats.

The eyes:
The condition of the eyes of the animals before the administration of alloxan, during the subsequent period and just before the animals were sacrificed as well as the data on the microscopical post-mortem examination, were recorded.
Fig. 2.
A - Glomerulus of control rat. Basal membranes of normal thickness (× 900).  
B - Glomerulus of an insulin deficient rat: hyaline swelling of the basal membranes, which are otherwise normal. (× 900).

by ophthalmologists. For microscopic examination the right eye of each animal was embedded in nitrocellulose and sectioned in a frontal plane, whereas for the left eye, the paraffin technique was employed, with sectioning in a sagittal plane. All eyes were fixated in formalin, 4 %/6. Every 5th section of each eye was stained, the nitrocellulose group alternatingly with haematoxylin-eosin and the van Gieson method, the paraffin group only with haematoxylin-eosin. To avoid bias, the ophthalmologist did not know which of the animals were insulin deficient.

At the beginning of the experiment all animals had normal eyes. During the experiment a cataract of different density developed in 4 rats. In the 4 other rats only a slight lens sclerosis developed. As long as their fundus was visible, no animal showed any retinal change.

The microscopic examination revealed that haemorrhages had occurred in none of the eyes. Signs of sclerosis of both smaller and larger vessels were not seen, not even thickening of basal membranes. There were no micro-aneurysms.
Table 2.

<table>
<thead>
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<th></th>
<th>8178 R</th>
<th>8179 R</th>
<th>8181 R</th>
<th>8184 R</th>
<th>8185 R</th>
<th>8186 R</th>
<th>8189 R</th>
<th>8193 R</th>
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<tr>
<td>Cataract</td>
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<td>Pathology of the retina:</td>
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<td>Haemorrhages</td>
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<td>Degenerative foci</td>
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<td>Other kind of foci</td>
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<td>Vitreous</td>
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<td>exudate</td>
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<td>Chorioidea changes</td>
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<td>congenital vessel anomaly</td>
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<td>Changes in other parts of the eye</td>
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<tr>
<td>Insulin deficiency</td>
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As is shown in Table 2, two of the alloxan-negative rats showed some inflammatory change:

No. 8181 had a small focus of some macrophages in the right retina.

8185 showed, bilaterally, small, mostly perivascular infiltrations of »lymphocytes« and some »lymphocytes« and »plasmacells« in the vitreous.

One of the insulin deficient rats (No. 8189) showed bilaterally a congenital vascular anomaly. The central artery and vein of the optic nerve did not enter the retina, but branched at the level of the lamina cribrosa into a vascular pattern, similar to that of the retinal vessels, which is why this was not observed clinically. They followed a course through the sclera, where small anastomoses with the retinal capillaries were seen. In the retina no rods and cones, and no ganglioncell-layer had developed.

Summarising it may be stated, that no ocular change could be seen, which would usually be described as a diabetic retinopathy.

SUMMARY

The problem arises, whether the late complications of diabetes are due to insulin deficiency. In spite of an intensive search in the literature, we could not find any convincing experimental or clinical evidence.

If it is not proved, that late diabetic complications are caused by insulin deficiency, the question remains whether the whole clinical picture of diabetes is accounted for by insulin deficiency only or whether this is merely a part of the syndrome: diabetes.

In order to make some contribution to this essential and fundamental problem of diabetes we tried to demonstrate pathological changes in rats, which were made alloxan insulin deficient for a period of a year.

We were not able to observe any changes in the livers, kidneys, eyes and larger and small vessels of these animals, comparable to those observed in human diabetes.

This investigation therefore gives no support to the general and commonly accepted conception, that, at least in the rat, insulin deficiency is responsible for the development of late diabetic complications.

REFERENCES


Poulsen, J. E.: Diabetes 2, 7, 1953.