ON THE INDUCTION OF DIABETES
BY MEANS OF ALLOXAN

By J. H. GAARENSTROOM, From the Pharmacological Department,
University of Leyden, Holland

(Received 29 July 1946)

It was observed by Dunn, Sheehan & McLetchie [1943] that the urine of animals
injected with alloxan contains a considerable amount of sugar. This phenomenon
became the object of an extensive study by these and other investigators, whose
results have been reviewed [Liebmann, 1944; Ingle, 1945; Chen, 1945]. A single
injection of alloxan in rats, rabbits and other animals causes, to begin with, a tran-
sient but pronounced hypoglycaemia, followed in 12–48 hr. by more or less marked
symptoms of diabetes, i.e. glycosuria, polyuria and high blood-sugar values. At
the same time the β-cells of the islets of Langerhans, which are looked upon as centres
for the production of insulin, are seen to degenerate. The transient attack of hypo-
glycaemia may be due to the release of the total amount of insulin stored in the
pancreas, and this release, therefore, may be regarded as the primary effect of the
injury caused by the alloxan.

Our principal object in this study has been the elucidation of the way in which
this process is influenced by the hypophysis in rats. The results obtained in the
initial stage of this study have already been published [Gaarenstroom & de Jongh,
1946]. A detailed account of our later experiments is given below.

METHODS

The male and female rats used in the experiments weighed 100–160 g. and could eat
and drink freely. On the 4th day of the experiment some of the animals received a
subcutaneous injection of 10–25 mg. of alloxan dissolved in 0.15–0.35 ml. of water.
The attack of hypoglycaemia, which should follow the injection, was prevented by
administering by stomach tube 3 ml. of a 20% dextrose solution 45 min. beforehand.
When the injection was ineffective, it was repeated the next day. Dose and treat-
ment were varied somewhat according to the amount of sugar found in the urine.
Four days later, i.e. on the 8th day of the experiment, the hypophysis was removed
in about half the injected animals. Of the animals which had not been injected, about
half were also hypophysectomized.

During four periods of 3 days each, the urine excreted by the animals which had
been injected with alloxan was collected, and its amount and sugar and nitrogen
contents determined: sugar by the titration method according to Benedict, and
nitrogen by means of the micro-Kjeldahl method. The 3-day periods extended from
the 1st to the 3rd, from the 6th to the 8th, from the 9th to the 11th, and from the
12th to the 14th days; the first set of estimations, therefore, was made before the
alloxan injection, the second after the latter but before hypophysectomy, and the
third and fourth after this operation. In addition, on the 8th and 14th days blood-
sugar values were determined before and after the administration of a 20% dextrose
solution. The latter was given as an oral dose of 3 ml. 2 hr., together with an intra-peritoneal injection of 2 ml. administered 1 hr., before the determination of the blood-sugar value. The blood-sugar values were determined by the Hagedorn-Jensen method. The changes in body weight too were noted, and in some of the animals the amount of acetone in the urine was estimated.

Of the hypophysectomized control animals, i.e. those that did not receive an alloxan injection, the urine was collected during two periods of 3 days in order to determine its nitrogen content. These figures served for comparison with the corresponding ones obtained from rats injected with alloxan. Finally blood-sugar values were determined before and after the administration of dextrose.

Of the non-hypophysectomized control animals too the blood-sugar value was estimated, but no attempt was made to estimate the nitrogen content of the urine, as the latter would not have differed from that found in the urine of the test animals in the period before the alloxan injection.

Chemical estimations were not considered reliable unless duplicate values obtained from two different samples were found to agree. The difference between the duplicate values rarely exceeded 10 % in case of the urinary dextrose determinations, 10 mg. per 100 ml. in case of the blood-sugar estimations, and 20 mg. in case of the nitrogen determinations. If they did, the determinations were rejected. The efficacy of the hypophysectomy was tested by histological examination of the sella turcica.

RESULTS

General

The administration of alloxan and the hypophysectomy caused a number of casualties: the mortality due to the injection varying between 10 and 20 %, and that caused by the gland extirpation between 30 and 40 % of the animals. Data obtained from animals succumbing before the 12th day of the experiment were rejected.

About 50 % of the animals to which alloxan was administered reacted with either no sugar-excretion or with very little. Those that excreted in the second period (as stated above, alloxan was administered in the interval between the first and the second periods) 2 g. of sugar or less were placed in a separate group. In this way six groups were formed: (1) animals injected with alloxan excreting more than 2 g. of sugar; (2) similarly treated animals excreting less than 2 g.; (3) animals that were not injected; (4) injected and hypophysectomized animals excreting more than 2 g.; (5) similarly treated animals excreting less than 2 g.; and (6) hypophysectomized animals that had not been injected.

Each group consisted of 8–16 rats. The results are summarized in Table 1. To save space the figures are not given in full but are represented by their mean values and ranges.

Sugar excretion in the urine

Rats that have not been treated with alloxan do not excrete dextrose, or, if they do, the amounts are negligible. In those cases where the amounts have been determined, they are, therefore, not mentioned in the table. After the administration of alloxan the rats that reacted excreted on the average 1·5–2 g. of sugar daily (4·5–6 g. in a period). One rat excreted as much as 3–4 g. daily. The sugar excretion reached its maximum in the second period, after which it tended to decrease. The group of rats
### Table 1

#### Non-hypophysectomized rats

<table>
<thead>
<tr>
<th></th>
<th>Alloxan injected</th>
<th></th>
<th>No treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High sugar</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>excretion group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Low sugar</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>excretion group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. of animals</strong></td>
<td>11</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td><strong>Body weight (g.)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>124 (99-152)</td>
<td>137 (120-154)</td>
<td>—</td>
</tr>
<tr>
<td>4th day</td>
<td>—</td>
<td>134 (118-149)</td>
<td>—</td>
</tr>
<tr>
<td>8th day</td>
<td>108 (84-126)</td>
<td>120 (107-134)</td>
<td>—</td>
</tr>
<tr>
<td>11th day</td>
<td>115 (88-137)</td>
<td>124 (102-144)</td>
<td>—</td>
</tr>
<tr>
<td>14th day</td>
<td>101 (70-118)</td>
<td>121 (112-134)</td>
<td>132 (104-167)</td>
</tr>
<tr>
<td><strong>Amount of urine (ml.)</strong>:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st period</td>
<td>8 (4-11)</td>
<td>12 (7-23)</td>
<td>—</td>
</tr>
<tr>
<td>2nd period</td>
<td>66 (32-134)</td>
<td>40 (25-64)</td>
<td>—</td>
</tr>
<tr>
<td>3rd period</td>
<td>88 (40-156)</td>
<td>36 (21-80)</td>
<td>—</td>
</tr>
<tr>
<td>4th period</td>
<td>78 (21-156)</td>
<td>29 (10-66)</td>
<td>—</td>
</tr>
<tr>
<td><strong>Sugar in urine (g.)</strong>:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd period</td>
<td>4·5 (2-0-11-0)</td>
<td>0·8 (0-0-1·6)</td>
<td>—</td>
</tr>
<tr>
<td>3rd period</td>
<td>6·1 (2-6-10-4)</td>
<td>0·8 (0-0-3·1)</td>
<td>—</td>
</tr>
<tr>
<td>4th period</td>
<td>5·4 (9-6-8·2)</td>
<td>0·3 (9-0-2·6)</td>
<td>—</td>
</tr>
<tr>
<td><strong>Blood-sugar values (mg. per 100 ml.)</strong>:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8th day: Fasting</td>
<td>98 (62-145)†</td>
<td>94 (64-123)</td>
<td>—</td>
</tr>
<tr>
<td>After glucose</td>
<td>348 (330-375)</td>
<td>377 (238-540)</td>
<td>194 (90-378)</td>
</tr>
<tr>
<td></td>
<td>421 (346-565)</td>
<td>356 (150-567)</td>
<td>104 (90-148)</td>
</tr>
<tr>
<td>14th day: Fasting</td>
<td>116 (60-226)</td>
<td>92 (69-118)</td>
<td>74 (54-83)</td>
</tr>
<tr>
<td>After glucose</td>
<td>350 (247-490)</td>
<td>273 (173-427)</td>
<td>116 (86-182)</td>
</tr>
<tr>
<td></td>
<td>387 (277-528)</td>
<td>321 (167-536)</td>
<td>170 (102-358)</td>
</tr>
<tr>
<td><strong>Nitrogen in urine (mg.)</strong>:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st period</td>
<td>208 (112-294)</td>
<td>266 (134-375)</td>
<td>—</td>
</tr>
<tr>
<td>2nd period</td>
<td>519 (336-908)</td>
<td>354 (173-464)</td>
<td>—</td>
</tr>
<tr>
<td>3rd period</td>
<td>595 (411-1000)</td>
<td>494 (274-813)</td>
<td>—</td>
</tr>
<tr>
<td>4th period</td>
<td>510 (137-783)</td>
<td>386 (206-472)</td>
<td>—</td>
</tr>
</tbody>
</table>

* During 1st period.  
† Estimated in only 4 animals.

#### Hypophysectomized rats

<table>
<thead>
<tr>
<th></th>
<th>Alloxan injected</th>
<th></th>
<th>No treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High sugar</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>excretion group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Low sugar</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>excretion group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No. of animals</strong></td>
<td>11</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

**ALLOXAN DIABETES**
that did not react, i.e. those that during the first period did not lose more than 2 g. of sugar, showed a similar behaviour, although the top of the curve in this case was, of course, much lower.

When the animals which had been treated with alloxan were hypophysectomized, the sugar excretion showed a sharp fall. Of the 9 rats which in the period before the extirpation together had excreted 45-2 g. of dextrose and were still alive at the end of the experiment, three only continued to excrete sugar in the '4th period', and the total amount produced in the latter was 5-1 g. only. In the animals which had reacted weakly to the alloxan injection, the sugar excretion almost immediately fell to zero after removal of the pituitary.

**Blood-sugar values**

In the animals injected with alloxan the fasting blood-sugar value appeared to be increased: whereas in normal rats an average value of 74 mg. per 100 ml. was found, the mean values observed in the different groups of injected rats were respectively 92, 94, 98, 104, 116 and 194 mg. per 100 ml. In the injected rats which showed no high sugar excretion, the values were on the whole lower but the difference was small. In the injected rats removal of the hypophysis led to very low values, namely 49 and 52 mg. per 100 ml. In the hypophysectomized control animals a similar value was found: 52 mg. per 100 ml.

After the administration of sugar the blood-sugar level increased as a rule to values of 250 mg. per 100 ml. or more.* It should be noted that this increase was independent of the presence of the hypophysis. In the injected animals which showed a high sugar excretion, the blood-sugar value was somewhat higher than in the injected animals which reacted with low excretion, but the difference was not considerable. In all the injected animals the blood-sugar values were markedly higher than in the animals which had received no alloxan. Among the latter the normal rats showed an increase which lagged behind that observed in hypophysectomized ones.

**Amount of urine**

In the animals which had been injected with alloxan, the amount of urine showed a marked increase: it was, in fact, sometimes more than ten times as large as before the injection. There is, therefore, some relation between the increase of the amount of urine and the excretion of sugar, but certainly there is no proportionality between the two. The animals which reacted weakly to the alloxan, and even those that excreted no sugar at all, showed a fairly strong polyuria. The removal of the hypophysis had hardly any effect on the amount of urine excreted by animals that reacted positively to the alloxan injection, whereas in animals that reacted weakly or not at all, an increase was noted. In the hypophysectomized animals which had received no alloxan, the amounts were nearly the same.

**Excretion of nitrogen**

The administration of alloxan markedly increased the nitrogen content of the urine. This appeared to be a general rule. The increase of the nitrogen value in the rats which reacted with a high excretion of sugar amounted on the average to 265%.

* In our earlier experiments, of which the results have been published elsewhere, a smaller amount of sugar was administered, and the increase of the blood-sugar level accordingly was less striking.
and to 184% in those that reacted weakly or not at all. A comparison between the nitrogen content of urine excreted by the control animals after extirpation of the hypophysis, with that of the urine excreted by the test animals before they had been injected, shows that hypophysectomy too causes an increase. As alloxan injection and hypophysectomy affect the nitrogen excretion in a similar way, it will be difficult to decide what part of the increase is to be assigned to each of them when they are acting simultaneously.

Body weight

After the administration of alloxan the body weight showed, at least in the beginning, a decrease. Between the latter and the extent of the sugar excretion, there appeared to be a certain parallelism. Hypophysectomy affected the body weight but little.

Ketosis

Acetone was never found in the urine of rats injected with alloxan.

DISCUSSION

The results of our experiments confirm and extend those obtained by the investigators of other countries in their study of the diabetes induced by alloxan. They are also of importance for our knowledge of the patho-physiology of diabetes in general.

In the first place our experiments allow us to draw some conclusions with regard to the cause of the increased nitrogen excretion in diabetes. This increase, which in the figures given above is clearly perceptible, must be the result of an intensified protein decomposition but this may be attributed to various causes. The most plausible supposition would be that the decrease in the consumption of carbohydrates makes it impossible to obtain the required amount of energy in the ordinary way, and therefore necessitates the decomposition of other substances, such as proteins and fat. It is also possible that insulin favours the synthesis of proteins, and that the latter therefore decreases because of the damage done to the pancreas. Finally, the so-called glyconeogenesis from proteins, i.e. the transformation of proteins into carbohydrates might, in case of an insulin deficit, be intensified. All three possibilities have been advocated [cf. the reviews by Esveld, 1942; Marx & Evans, 1944; and Lukëns, 1944]. None of these possibilities is actually excluded by our experiments though, as even in those animals that excreted little or no sugar the nitrogen excretion was markedly increased, the latter cannot be due to low level of carbohydrate consumption alone; it must be assumed that the insulin deficit acts in another way also, either as a check to protein synthesis or as an accelerator of the transformation of proteins into carbohydrates.

Our experiments also shed some light on the decrease which the sugar excretion of diabetic animals undergoes when the hypophysis is removed. This phenomenon, which in our experiments too was clearly perceptible, was first noted in 1930 [Houssay, 1942]. Though several hypotheses for the elucidation of this effect have been brought forward in which a number of hormones produced by the hypophysis and acting on the carbohydrate metabolism are suggested as playing a part, no fully satisfactory explanation has as yet been given. Owing to technical difficulties the various possibilities could not be tested. Pancreas extirpation could not be carried out satis-
factorily in animals of a smaller size than cats or dogs, and the latter were not available in sufficient numbers. Moreover, hypophysectomy is in these animals very difficult, and the effects of both operations are so serious that it is difficult to keep the animals alive. The induction of diabetes by means of alloxan, therefore, is an important acquisition, for it allows the use of small animals, which are more resistant and more easily hypophysectomized, and of which larger numbers are available, so that serial experiments can be carried out.

The decrease in sugar excretion after hypophysectomy might be ascribed to various causes. The most plausible ones are:

1. that less food is taken, and therefore less sugar absorbed;
2. that the consumption of sugar in the body, which is greatly reduced after the alloxan injection, resumes its former value;
3. that the transformation of protein, and perhaps that of fat too, into carbohydrates, which may have been enhanced by the alloxan treatment, undergoes a decrease.

The first cause certainly cannot be neglected. The hypophysectomized animals eat, as a rule, but little, and the amount of carbohydrates which they absorb is accordingly small. This must, of course, affect the dextrose excretion. Experiments in which the food consumption of hypophysectomized and non-hypophysectomized animals injected with alloxan is being studied are at present under way. Apart from the fact that the animals eat less, we have to consider the possibility that less sugar enters the circulation because the absorption from the intestinal tract is unfavourably affected by the removal of the hypophysis. The reduction of the amount of carbohydrates which the animals absorb, however, cannot be the sole cause of the decrease in sugar excretion. The fact that blood-sugar values which were determined when the injected animals had fasted 16 hr. or longer (and so may be regarded as nearly independent of the amount of food previously consumed) proved to be considerably increased in non-hypophysectomized animals and distinctly decreased when the hypophysis was removed, suggests the presence of an endogenous cause in addition to the reduction of the amount of sugar absorbed by the animals.

That the consumption of sugar in the body should be accelerated after hypophysectomy has often been claimed [cf. Russell, 1938, 1942, 1943], but this assumption finds no support in our experiments. The very high values of the blood-sugar level observed in hypophysectomized alloxan-treated animals after the administration of sugar are a strong argument against this view. That this increase does not lag behind the one observed in non-hypophysectomized animals injected with alloxan proves that the removal of large amounts of sugar suddenly brought into the circulation is in both cases a very difficult task. In rats that are neither injected nor hypophysectomized, the increase is much smaller.

As the second cause can be excluded, we must assume that the decrease of sugar excretion after hypophysectomy is due at least in part to the third: a decrease of the rate with which proteins are transformed into carbohydrates. This view has been advocated already by others, and various arguments in favour of it have been collected [cf. Long, 1943; Marx & Evans, 1944]. Until now, however, the possibility that the decrease in the sugar excretion might become entirely due to the two other causes has never been excluded.
ALLOXAN DIABETES

Another phenomenon which deserves our attention is the decrease in sugar excretion during the course of the experiment. In this we might see a tendency to recover. The presence of such a tendency showed itself clearly in four rats of which we studied the dextrose excretion during two periods of 3 days, namely, immediately after the alloxan injection and 20 days later: during the first period the total amount appeared to be 13.9 g., whereas in the second it was 1.7 g. only, a decrease of nearly 90%. This clearly demonstrates the tendency of the injured pancreas to recover spontaneously, which is the more remarkable as in cases of spontaneous diabetes in man the tendency is in the opposite direction. Should this mean that the injurious agent in man, in contrast to the agent in our experiments, remains constantly active? In that case it would be appropriate to look for this agent with the utmost energy. This explanation, however, is an improbable one, as a permanent diabetes can be induced by a short series of injections with a diabetogenic pituitary extract. Presumably the injury caused by the alloxan injection is of a less irreversible character than that which in human pathology causes diabetes.

The polyuria observed in rats injected with alloxan appeared to be only loosely connected with the excretion of dextrose, for even in animals which excreted no sugar the amount of urine was increased though to a lesser extent. So the diabetic polyuria may be not merely a consequence of the sugar excretion but due to an injurious effect of alloxan on the kidneys. According to Dunn et al. [1943], after administration of alloxan an injury in the kidneys was indeed revealed by histological examination, but it is uncertain whether this injury is responsible for the polyuria. The renal function does not seem to be impaired too seriously in our rats as the excretion of nitrogen had increased. That the disappearance of the polyuria coincides with a decrease in the sugar excretion, might be regarded as an indication of the presence of a relation of some kind with the activity of the pancreas. This problem will have to be studied more closely.

The character of the diabetes induced in our experiments by the administration of alloxan cannot be called severe. As the amount of food which the animals ate daily was not determined, we are unable to say how much carbohydrate they still consumed. The loss in body weight was only moderate and was, moreover, restricted to the first few days after the alloxan injection; the fasting blood-sugar values were not exceedingly high, and acetone was never found in the urine. However, it should not be forgotten that the animals which died shortly after the alloxan administration were not taken into account and may have had a diabetes of a more serious character. In the literature dealing with diabetes induced by alloxan cases with lethal effect have been often reported.

SUMMARY

Subcutaneous administration of alloxan (one or two injections of 100–200 mg. per kg. body weight) causes in about half the number of injected rats a considerable excretion of sugar. The fasting blood-sugar level rises above that of normal rats, and after giving dextrose orally and intraperitoneally, the increase of the blood-sugar values is very great. A polyuria and an increase in the nitrogen content of the urine are manifested at the same time.

In those rats that do not react to the alloxan injection by the excretion of considerable amounts of sugar, the blood-sugar level nevertheless shows similar deviations
from the normal, and in these animals too the amount of urine and its nitrogen content are increased.

The extirpation of the hypophysis causes a marked decrease or even the total disappearance of the sugar excretion. The fasting blood-sugar level sinks below that of normal rats, and is not more than that of hypophysectomized rats which did not receive alloxan. After the administration of sugar, however, the blood-sugar level of hypophysectomized rats injected with alloxan reaches a very high value, as high as that of non-hypophysectomized rats treated in the same way. The polyuria and the increased nitrogen content of the urine persist after the extirpation of the hypophysis, but values of the same order of magnitude were found in hypophysectomized control rats.

I am grateful to Miss C. C. Polder for technical assistance.

REFERENCES