STUDIES ON INTRAVENOUS GLUCOSE TOLERANCE CURVE

4. EXPERIMENTS ON NORMAL AND ADRENALECTOMIZED RATS

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There are many works on change in the blood sugar values following the intravenous administration of glucose (Thaunhauser and Pfitzer, 1913; Tunbridge and Allibone, 1940; Samuels et al., 1937 a, b; Greville, 1943; Soskin, 1944; Nonohe, 1952; Amatuzio, 1953; Hirata, 1954; Scow and Cornfield, 1954 a, b). It is generally known that the pituitary, adrenocortical and thyroid hormones concerned with carbohydrate metabolism. At present, however, the effect of these hormones upon the glucose tolerance is not completely elucidated. Recently, Takeuchi et al. (1957) and Fukushima (1957 a, b) made experiments on this problem with dogs and rabbits, respectively, and reported that the so-called atypical glucose tolerance curve is not the least rare, and that the type of the curve is dependent on a certain metabolic equilibrium in the living body, especially in the endocrine system. In the present experiment the author observed extremely various atypical curves in rats, which can not only be attributed to individual difference in ability to assimilate injected glucose, but which is likely dependent on the metabolic equilibrium, especially on the endocrine equilibrium. Accordingly, he carried out experiments on the relation between the blood sugar curve and the adrenal function, and obtained highly affirmative results.

MATERIAL AND METHODS

Sixty male rats of Wistar strain weighing about 200 g and fed on standard diet (Oriental Co., MC 5) were used. In 26 of them, bilateral adrenalectomy was performed under ether anesthesia according to the technique described by Ingle and Griffith (1949). Further, 9 of these 26 animals were divided into 3 groups composed of each 3 animals, and from the 4th day after the operation, all were subcutaneously injected on the back with hydrocortisone acetate (Hydrocortone Acet., Merck) for 2 days, in a daily dose of 0.25 mg, 0.5 mg and 2.5 mg according to the group. The glucose tolerance test was made 4 hours after the last injection. The test was performed according to the method by Samuels et al. (1937a) with the normal (60 cases), adrenalectomized (6 cases one week and 20 cases 3 days after the operation) and hormone administered (9 cases). After 4 hours fast, the animals were intravenously given in the saphenous vein 1.25 g/kg of a 50% glucose solution. Before and 10, 20, 30, 60, 90 and 120 mins. after the injection, each 0.02 cc of samples were taken from a tail vein, and the blood sugar value was determined by the King and Garner method (1947).

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RESULTS

I. Observation on non-treated rats

The mean value for blood sugar of 60 normal rats at each bleeding time is presented in Table I and Fig. 1. At 2 hours after glucose injection the values were still higher than that of pre-injection. The standard deviation at different bleeding times were not always uniform, being relatively larger at 10 and 30 mins. after the injection. The obtained curve were classified according to their characteristics into 4 types reported previously by Takeuchi (1957) and Fukushima (1957).

Exponential type (Type I): To the curve of this type the equation of monomolecular reaction is applied. The calculated constants from the blood sugar values at 20 min. intervals were the following: e = 0.67, c = 75 (Fig. 2).

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Before injection</th>
<th>10</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>86 ± 10</td>
<td>262 ± 39</td>
<td>228 ± 32</td>
<td>154 ± 27</td>
<td>127 ± 15</td>
<td>111 ± 13</td>
</tr>
</tbody>
</table>

Table 1. Blood sugar level after the intravenous injection of glucose

Fig. 1. Glucose tolerance curve in normal rats. Standard deviation shown on either sides of mean blood sugar values.

↓: Intravenous injection of 1.25 g/kg of glucose

Fig. 2. Exponential type (1)

↓: Intravenous injection of 1.25 g/kg of glucose
Humped curve type (Type II): This is considered to be a overlapping form of type I and the so-called humping curve, which appears in many cases at 60-90 mins. after glucose administration (Fig. 3).

Sigmoid type (Type III): An extremely slow fall appears from 10 to 30 mins. after glucose administration and later the curve is similar to type I (Fig. 4).

Straight line type (Type IV): In cases of this type, blood sugar value changes linearly according to the bleeding time. Linear curves, not small in number, in which the fall rate is greater from 10 to 60 mins. after glucose administration, and then smaller were conveniently grouped under this type (Fig. 5).

Table 2 shows all the observed cases classified into these 4 types. To type III belonged the largest number of cases, 26 of 60 cases (43%), followed by 15 cases of type I (25%) and 14 cases of type II (23%). To type IV belonged only 5 cases (8%). The average blood sugar value for each type at each bleeding time is shown in Table 3. There was no remarkable difference between the 4 types in the blood sugar values before glucose administration and at 30, 90 and 120 mins. after the administration. However, at 10 mins. after the administration, the values in types I and IV were higher than those in types II and III. And the value at 60 mins. was higher in type II than in the other three.
Table 2. Observed frequency in types of glucose tolerance curve in normal rats

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Exponential type (I)</th>
<th>Humped curve type (II)</th>
<th>Sigmoid type (III)</th>
<th>Straight line type (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>15</td>
<td>14</td>
<td>26</td>
<td>5</td>
</tr>
<tr>
<td>(25.0%)</td>
<td>(23.3%)</td>
<td>(43.3%)</td>
<td>(8.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Blood sugar level in 4 types of glucose tolerance curve in normal rats

<table>
<thead>
<tr>
<th>Type</th>
<th>Before injection</th>
<th>Mean blood sugar level (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time after injection of glucose (mins.)</td>
<td>10</td>
</tr>
<tr>
<td>Exponential</td>
<td>94 ± 10</td>
<td>298 ± 53</td>
</tr>
<tr>
<td>Humped curve</td>
<td>84 ± 8</td>
<td>253 ± 33</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>92 ± 9</td>
<td>254 ± 39</td>
</tr>
<tr>
<td>Straight line</td>
<td>86 ± 13</td>
<td>280 ± 70</td>
</tr>
</tbody>
</table>

II. Observations on Adrenalectomized Rats
A: Adrenalectomized rats
1) One week after the operation: Blood sugar curves for 6 cases represented...
in Fig. 6. Except one case all showed abrupt linear fall, and conspicuous hypoglycemia at 60-90 mins. after the glucose injection. Two animals died after convulsion at 90-110 mins.

2) Three days after the operation: Average blood sugar values for 20 cases, at each bleeding time, and their curves are presented in Table 4 and Fig. 7. Except for 2 cases, all pre-injection values were a little lower than those for the same animals before the operation; that is, the average was 89±7.8 mg/dl, the normal being 94±9.9 mg/dl (t=2.55). After the glucose injection, the curve showed an abrupt fall, attaining the pre-injection level in the most cases. The average blood sugar value for 20 cases showed a decrease of about 48 mg/dl at 10 mins. after the injection compared with the pre-operative level, and the decrease was most prominent at 30 and 60 mins. The standard deviation at each bleeding time was smaller than that in the normal. The type of the curve was investigated with each individual case, and all of them were found to belong to type I, and the diversity of type as seen in the normal state was not observed.

Table 4. Blood sugar level in adrenalectomized rats 3 days after the operation

<table>
<thead>
<tr>
<th>Time after injection of glucose (mins.)</th>
<th>Before injection</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before adrenalectomy</td>
<td>94±10</td>
<td>263±35</td>
<td>--</td>
<td>230±25</td>
<td>153±23</td>
<td>124±14</td>
<td>113±13</td>
</tr>
<tr>
<td>After adrenalectomy</td>
<td>89±8</td>
<td>215±17</td>
<td>123±18</td>
<td>115±19</td>
<td>89±10</td>
<td>84±10</td>
<td>80±9</td>
</tr>
</tbody>
</table>
Fig. 7. Glucose tolerance curve in adrenalectomized rats 3 days after the operation. Standard deviation shown on either side of mean blood sugar value.

↓: Intravenous injection of 1.25 g/kg of glucose

**B**: Adrenalectomized rats treated with hydrocortisone acetate

Two cases which showed type II and one case which showed type III before the adrenalectomy all came to show type I. The administration of 0.25 mg of hydrocortisone acetate scarcely altered the curve type. Fig. 8a shows a representative case. The descending curve at 10–30 mins. after glucose injection was very similar to that after the adrenalectomy. Thereafter the curve became nearly horizontal, and did not fall below the pre-injection level. And the humping curve which could be seen before the adrenalectomy had disappeared, presenting approximately type I. Two of 3 cases to which was administered daily 0.5 mg showed type
Fig. 8. Effect of administration of hydrocortisone acet. on glucose tolerance curve: in daily dose of 0.25 mg (a), 0.5 mg (b) and 2.5 mg (c) for 2 days. ↓: Intravenous injection of 1.25 g/kg of glucose

III and the other one type I before the adrenalectomy, but all came to present type I after the operation. Fig. 8b exhibits the curve for one case which showed type III before the operation. After the hormone administration the curve for the time period 10–60 mins. was approximately similar the corresponding part obtained after the operation and before the administration of the hormone. Thereafter the curve descended in parallel with the pre-operative one. However, at 2 hours after glucose injection, the value was lower than before the injection. Of 3 cases for the daily dose of 2.5 mg, 2 showed type III and one type I before the operation, but all turned into type I after the adrenalectomy, again recovering the initial type after the administration of the hormone. The values at each bleeding time almost agreed with the pre-operative one. Fig. 8c represents the curve of one case which showed type III before the adrenalectomy.

DISCUSSION

Takeuchi et al. (1957), who investigated the frequency of the so-called atypical glucose tolerance curve for the dogs, reported 1/3 for the adult and 2/3 for the young. Fukushima (1957) found out high frequency of such curve for the rabbits, and reported that 2/3 of them belonged to the humped curve type. According to Tunbridge and Allibone (1940), 12 of 92 examined human cases showed the humped curve type and 3 the straight line type. In the present experiments with 60 rats, 75% displayed atypical curves, of which about 1/2 belonged to type III. And the descending rate of blood sugar values in all the observed cases was smaller than that reported for the human case and the dog, and approximately equal to that for the rabbit. Also in cases of type I, the constant e^(-k) for the descending rate was smaller than that for the rabbit. In this way the frequency of the type
of the glucose tolerance curve varied from one animal kind to another, and in the same type the descending rate differed from one another. One of the reasons for this may be attributed to difference in experimental condition, namely the doses of injected glucose (.5 k/kg for man, 1 g/kg for dog, 1.25 g/kg for rabbit, 1.25 g/kg in the present study), fasting time (17 h. for man, 12 h. for dog, 12 h. for rabbit and 4 h. in the present experiments), bleeding time (3 mins. after glucose injection and thereafter 10-20 mins. intervals for man, dog, rabbit; and 10, 20, 30 min. and later 30 min. intervals in the present experiments). Also difference intrinsically due to the kind of animal may not be denied. This point could not be clarified in the present experiments.

The mechanism of the occurrence of the atypical glucose tolerance curve is still in obscurity. Ross (1936) and Best (1934) attributed the atypical curve to the adrenal hyperfunction produced by the inadequate experimental procedure. However, Takeuchi et al. (1957) explained that the type of the curve would suggest the endocrinological equilibrium of the living body, because the type as well as its frequency differed in the adult and the growing stage, and because there is difference in insulin sensitivity index between cases with different curve types. Britton (1932), from the results of experiments with adrenalectomized cats estimated a relationship between the adrenocortical hormone and sugar metabolism. In view of these, the author undertook the observation of glucose tolerance curve for adrenalectomized rats. One week after the operation, all the 6 cases showed a linear fall, developing remarkable hypoglycemia. Before the operation, 3 of them showed type II, 2 type I and one type III, but all these characteristics disappeared after the operation. Twenty adrenalectomized cases showed, at 3 post-operative day, type I with relatively small fluctuations from one individual to another, pre-operative variation in the curve type having been completely disappeared. From these facts it can be estimated that diversity in the glucose tolerance curve may be due to individual difference in adrenal function, or more generally in the endocrine function. Subsequently, hydrocortisone acetate was administered to adrenalectomized rats, assuming that the substitution of the adrenal hormone would clarify this point. When the hormone was given twice in a single dose of 0.25 mg or 0.5 mg no significant result was obtained, but when 2.5 mg was given twice, the type I produced by adrenalectomy returned to the pre-operative type. It is a question to which of the many actions of hydrocortisone the above mentioned effect can be attributed. Is it related to the well known action on liver glycogen, or on glucose utilization in the muscular tissues? Concerning this, author has not yet sufficient data for explanation. In the present experiments, adrenalectomy did not produce any changes in the type in animals which showed type I, but it only increased the descending rate. This may be explained by the inhibitory action of the adrenal hormone for glucose uptake into the peripheral tissues. However, Fukushima (1957), analyzing the glucose tolerance curve in rabbit, noted the presence of a humping which had no relation with glucose utilization ability in the periphery. If the atypical curve for the rat is considered to be an overlapping one of type I and a humping, the participation not only of the muscle but also of the liver could not be denied. At any rate it seems highly probable that there may exist close connection between the diversity of glucose tolerance curve
and individual difference in adrenocortical function.

SUMMARY

Changes in blood sugar value elicited by intravenous administration of glucose were investigated with 60 normal rats. Forty five of the obtained curves (75%) belonged to the so-called atypical curve type.

Following adrenalectomy, the diversity in the curve type which was characteristic of the normal animal disappeared and the curves showed the exponential type with only slight fluctuations from one individual to another.

Administration of hydrocortisone acetate in a daily dose of 2.5 mg for 2 days after adrenalectomy returned the glucose tolerance curve to the pre-operative one.

REFERENCES