COMPARATIVE STUDIES OF ALLOXAN DIABETIC DOG AND PANCREATECTOMIZED DIABETIC DOG ON THE REGULATION OF THE CARBOHYDRATE METABOLISM WITH SPECIAL REFERENCE TO THE ACTION OF ACTH AND ADRENOCORTICAL HORMONES ON THE INSULIN REQUIREMENT

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Subjects of diabetogenic or contrainsulin action of ACTH, and cortisone in the regulation of carbohydrate metabolism were reported by numbers of workers (Long, Katzin and Fry, 1940; Grattan, Jensen and Ingle, 1941; Bodo, Kurtz, Sinkoff and King, 1952), while, the effect of DOC was also reported but with different results. Ingle and Thorn (1941) and Lászt (1947) assumed its action as contrainsulin effect, while, Köhler and Müller (1952) thought the action might be an insulin effect. On the effect on carbohydrate metabolism after the parenteral administration of ACTH and adrenocortical hormones, there were sufficient number of reports already made by the staffs of our laboratory.

It was found by Kajiyama (1955) that either ACTH or DOC possessed an insulin factor. Clinically, for the most diabetic patients, cortisone acted similarly as insulin (Nakano and his associates, 1955). ACTH seemed to control convulsive seizure due to insulin and tend to enhance glucose tolerance curve in adrenalectomized rabbits (Fujita, 1954). According to Wakabayashi’s experiments (unpublished data), either ACTH or cortisone seemed to increase the deposit of liver glycogen, however, DOC has tendency to decrease its glycogen deposit. Although the action of ACTH and adrenocortical hormones on the regulation of carbohydrate metabolism, particularly on insulin or contrainsulin action was extensively studied, but from what was reported, this problem seems rather complicated and still needs further research work. As to the experimental diabetes mellitus, several investigators such as Thorogood and Zimmerman (1945) reported the comparative studies between the alloxan diabetic dog and the pancreatectomized dog and found the difference of insulin requirement between the two types of diabetes in dogs.

The present investigation was undertaken for the purpose of determining the insulin requirement of these diabetic dogs by the method by Allan (1924) and MacBryde (1933), and also of finding the difference of the effect between ACTH
and adrenocortical hormones by their daily administration for a prolonged period, in order to confirm the action of these hormones upon the regulating mechanism of carbohydrate metabolism, especially of insulin and contrainsulin effects.

**EXPERIMENTAL METHOD**

For inducing alloxan diabetes to dog, alloxan 70mg per kg body weight was administered intravenously, and for the other dog, the total pancreatectomy was performed with the duodenum left intact, under the Isomytal (isomyl-ethylbarbiturate) anesthesia after fasting for 24 hrs. On the next day after removal of pancreas, the insulin treatment was started and the preparation known as Choliine was daily injected intramuscularly of 2ml, which contains 150mg choline chloride, 30mg inositol, 40mg DL-methionine, 4µg vitamin B₁₂.

For the determination of urinary sugar on hrs. specimen of the two types of diabetic animals Pavy-Kumagawa-Sudo method was used, and for measuring the fasting blood sugar value, the Hagedorn-Jensen method was applied. The insulin requirement was calculated from the urinary sugar value as previously reported by the author (1956). The effect of ACTH (ACTHar, Armour), cortisone (Cortone, Merck), and DOCA (Syncorta, Takeda) upon the insulin requirement of two types of diabetic dogs was observed.

**RESULTS**

1. The insulin requirement of alloxan and pancreatectomized dogs

   **(A) Insulin-urinary sugar equivalent**

   Various methods for determination of insulin requirement were used by many workers, but the estimation by insulin-urinary sugar equivalent method to be most practical for our experience, and from this equivalent the insulin dosage would be readily calculated.

   **Table 1. Insulin-urinary sugar equivalent in alloxan-diabetic and pancreatectomized dogs (mean values)**

<table>
<thead>
<tr>
<th>Cases</th>
<th>A (gm)</th>
<th>B (gm)</th>
<th>E (u)</th>
<th>(A-B)/E (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloxan diabetic dog</td>
<td>2</td>
<td>128.6</td>
<td>40.3</td>
<td>16</td>
</tr>
<tr>
<td>Pancreatectomized diabetic dog</td>
<td>2</td>
<td>32.5</td>
<td>7.3</td>
<td>9</td>
</tr>
</tbody>
</table>

   A: The average value of urinary sugar before insulin treatment
   B: The average value of urinary sugar after insulin treatment
   E: Dosage of insulin used per day
   (A-B)/E: Insulin-urinary sugar equivalent

   The Table 1 indicates that insulin-urinary sugar equivalent is approximately 5.5gm of glucose for one unit of insulin in alloxan diabetic dogs, and 2.8gm of glucose for one unit in pancreatectomized dogs. When calculated from the value of insulin-urinary sugar equivalent, the insulin requirement per day would be 23.4 unit for alloxan diabetic dogs, while 11.6 unit insulin would be required for pancreatectomized dogs.

   **(B) Insulin-blood sugar equivalent in alloxan diabetic and pancreatectomized dogs**

   The fasting blood sugar levels were compared between the two types of dia-
betes before and after insulin treatment under the condition that the animals were fed of the same diet. Observation was also made of the insulin effects on the fasting blood sugar levels following an increased dosage of the injection after the previous known dosage was given. It was shown that the fasting blood sugar levels in pancreatectomized dogs were found sensitive to insulin treatment. The influence on fasting blood sugar levels with or without insulin injection is shown in Table 2. On the contrary, the fasting blood sugar levels in alloxan diabetic dogs were found indifferent to insulin treatment. In other words it is noted that the elevation of blood sugar values could be prevented by the following insulin treatment in the pancreatectomized animals. While, in the alloxan dogs, the blood sugar demonstrated certain resistance to insulin, and little or no expected fall of blood sugar could be observed when an increased dosage of insulin were administered after some day's previous insulin treatment (Table 3).

### Table 2. Insulin-blood sugar equivalent in alloxan diabetic and pancreatectomized dogs (mean values)

<table>
<thead>
<tr>
<th>Cases</th>
<th>A' (gm)</th>
<th>B' (gm)</th>
<th>E' (u)</th>
<th>(A'-B')/E' (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloxan diabetic dogs</td>
<td>2</td>
<td>203</td>
<td>170</td>
<td>9</td>
</tr>
<tr>
<td>Pancreatectomized dogs</td>
<td>2</td>
<td>372</td>
<td>240</td>
<td>9</td>
</tr>
</tbody>
</table>

A': The average value of blood sugar before insulin treatment  
B': The average value of blood sugar after insulin treatment  
E': Dosage of insulin used per day  
(A'-B')/E': Insulin-blood sugar equivalent

### Table 3. Fasting blood and urinary sugar values during insulin treatment of different dosage in an alloxan diabetic dog

<table>
<thead>
<tr>
<th>Days after alloxan injection</th>
<th>Injected dose of insulin (u/day)</th>
<th>Fasting blood sugar values (mg%)</th>
<th>Urinary sugar values (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37-41</td>
<td>(-)</td>
<td>242</td>
<td>108.6</td>
</tr>
<tr>
<td>42-46</td>
<td>3X3</td>
<td>216</td>
<td>40.3</td>
</tr>
<tr>
<td>47-53</td>
<td>4X3</td>
<td>336</td>
<td>40.3</td>
</tr>
<tr>
<td>54-56</td>
<td>5X3</td>
<td>330</td>
<td>47.5</td>
</tr>
<tr>
<td>57-60</td>
<td>6X3</td>
<td>308</td>
<td>52.2</td>
</tr>
<tr>
<td>61-69</td>
<td>6X4</td>
<td>298</td>
<td>31.2</td>
</tr>
<tr>
<td>70-74</td>
<td>8X4</td>
<td>263</td>
<td>13.6</td>
</tr>
<tr>
<td>89-96</td>
<td>8X4</td>
<td>294</td>
<td>64.0</td>
</tr>
<tr>
<td>97-108</td>
<td>10X4</td>
<td>252</td>
<td>50.1</td>
</tr>
<tr>
<td>109-116</td>
<td></td>
<td>1274</td>
<td>57.0</td>
</tr>
<tr>
<td>117-124</td>
<td>10X3</td>
<td>353</td>
<td>54.5</td>
</tr>
</tbody>
</table>

II. Effects of ACTH and adrenocortical hormones on the fasting blood sugar levels and the urinary sugar values in insulin treating alloxan diabetic dogs

When the fasting blood sugar levels and glycosuria became practically stabilized by insulin treatment (three injections daily), along with administration of insulin, the alloxan dogs were injected of ACTH, cortione and DOC, respectively three times a day and the effects of hormones on hyperglycemia and glycosuria were observed, as summarized in Table 4. The fasting blood sugar as well as glycosuria were definitely elevated when ACTH or cortisone was administered three times a day
for a period of 7~10 days. However, DOC when similarly given showed the decrease in the level of blood sugar, but as far as urinary sugar was concerned, there was hardly seen any change in the insulin treated alloxan diabetic dogs.

Table 4. The effect of injection of ACTH, cortisone or DOC (each 3 mg/kg, daily) on the fasting blood sugar value and urinary content in alloxan diabetic dog (mean values of two cases each)

<table>
<thead>
<tr>
<th></th>
<th>Fasting blood sugar values (mg%)</th>
<th>Urinary sugar values (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before the treatment</td>
<td>During the treatment</td>
</tr>
<tr>
<td>ACTH</td>
<td>298</td>
<td>+26.8</td>
</tr>
<tr>
<td></td>
<td>Increased or decreased percentage</td>
<td>+5.5</td>
</tr>
<tr>
<td>Cortisone</td>
<td>219</td>
<td>+26.0</td>
</tr>
<tr>
<td></td>
<td>Increased or decreased percentage</td>
<td>+5.5</td>
</tr>
<tr>
<td>DOC</td>
<td>330</td>
<td>-30.6</td>
</tr>
<tr>
<td></td>
<td>Increased or decreased percentage</td>
<td>-5.0</td>
</tr>
</tbody>
</table>

The insulin-glucose equivalent for increased value of urinary sugar following ACTH and cortisone administration was also determined. In the case of ACTH administration, it required excessively 4.5 units of insulin, while, in the case of cortisone, 3.4 units of insulin was required excessively in alloxan diabetic dogs. The significant change of blood and urinary sugar values of alloxan diabetic dogs following the administration of ACTH and adrenocortical hormones would be the fasting blood sugar levels after the time of withdrawal of the hormones i.e., the fasting blood sugar levels were markedly influenced by the withdrawal of DOC, and it immediately returned to its initial value, but the elevated blood sugar levels during the administration of ACTH or cortisone still continued to remain the high level and failed to resume its initial value even after the withdrawal of hormones.

III. Effects of ACTH and adrenocortical hormones on fasting blood sugar levels and urinary sugar values in insulin treated pancreatectomized dogs

Table 5 shows changes of fasting blood sugar levels and urinary sugar values following the daily administration of ACTH, cortisone and DOC three times a day in the insulin treated pancreatectomized dogs. In the case of pancreatectomized dogs, both the fasting blood sugar level and glycosuria were increased notably by
ACTH or cortisone administration, but its decrease was observed when injected of DOC as in the cases of alloxan diabetic dogs.

Although ACTH and adrenocortical hormones showed the same effect on the blood and urinary sugar values in either group of animals during the administration of these hormones, the obvious difference between the two diabetes was the fasting blood sugar levels after the withdrawal of these hormones. It is noted that in the pancreatectomized dogs, in contrast to alloxan diabetic dogs, the elevated fasting blood sugar value during the administration of ACTH or cortisone had returned to the initial value since withdrawing these hormones.

Table 5. The effect of injection of ACTH, cortisone or DOC (each 3 mg/kg, daily) on the fasting blood sugar value and urinary sugar content in pancreatectomized dog (mean values of two cases each)

<table>
<thead>
<tr>
<th></th>
<th>Fasting blood sugar values (mg%)</th>
<th>Urinary sugar values (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before the treatment</td>
<td>During the treatment</td>
<td>After the treatment</td>
</tr>
<tr>
<td>ACTH</td>
<td>435</td>
<td>552</td>
</tr>
<tr>
<td></td>
<td>Increased or decreased percentage</td>
<td>+26.9</td>
</tr>
<tr>
<td></td>
<td>Increased or decreased insulin requirement (u)</td>
<td></td>
</tr>
<tr>
<td>cortisone</td>
<td>326</td>
<td>432</td>
</tr>
<tr>
<td></td>
<td>Increased or decreased percentage</td>
<td>+32.5</td>
</tr>
<tr>
<td></td>
<td>Increased or decreased insulin requirement (u)</td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>457</td>
<td>387</td>
</tr>
<tr>
<td></td>
<td>Increased or decreased percentage</td>
<td>-15.3</td>
</tr>
<tr>
<td></td>
<td>Increased or decreased insulin requirement (u)</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

It was shown that ACTH and cortisone seemed to give enhanced effect on hyperglycemia and glycosuria in both alloxan or pancreatectomized dogs, while DOC had lowering effect on them. From what was observed on the action of hormones upon the regulation of carbohydrate metabolism, both ACTH and cortisone seemed to have a contrainsulin effect, but DOC possessed an insulin effect. The fact that these two distinctly opposing actions present was very interesting for the author, firstly, the fasting blood sugar levels were practically indifferent to the daily treatment with insulin; secondly, the elevation of fasting blood sugar levels following the daily administration of ACTH or cortisone in the insulin
treated alloxan diabetic dogs continued their high levels for over 7 days from the time of withdrawal of the hormone. In pancreatectomized dog, however, the elevation of blood sugar levels following daily administration of the above mentioned hormones was found to be reduced by the withdrawal of them to their initial values on the contrary to the former type of diabetes.

Foregoing observation revealed the fact that blood sugar levels of pancreatectomized dogs were immediately influenced by the withdrawal of ACTH or cortisone, while blood sugar level in alloxan diabetic dogs had more lasting influence than the former when the hormones were withdrawn. It could be interpreted that the indifference to insulin treatment and an intensive response to fasting blood sugar levels when treated by ACTH, or cortisone as observed in alloxan dogs might be due to the result of enhanced production of contrainsulin action in the endocrine system.

SUMMARY

1. Insulin-urinary sugar equivalent for one unit of insulin was found as 5.5gm of glucose in alloxan dog and as 2.8gm glucose in pancreatectomized dog. The daily dosage of insulin requirement was calculated as 23.4 units for alloxan diabetic and 11.6 units for pancreatectomized dog.
2. The fasting blood sugar level of pancreatectomized dog showed definitely high sensitivity to the action of insulin, but no sensitivity was demonstrated in alloxan diabetic dog.
3. The levels of blood sugar and glycosuria were elevated and the higher insulin dosage was required when ACTH or cortisone was administered, but the less dosage of insulin was required in the case of DOC. Also it was clarified that, under the experimental condition, ACTH and cortisone have a contrainsulin effect and DOC showed an insulin effect.
4. The elevated blood sugar level by the daily administration of ACTH and cortisone continued its high level for over 7 days from the time of withdrawal of the hormones in alloxan diabetic dogs, while blood sugar value was lowered immediately after the withdrawal of the hormones in pancreatectomized dogs, though concerning glycosuria, there was no difference noted between the two types of diabetes.
5. Significant difference was found that the fasting blood sugar level in alloxan diabetic dog was indifferent to insulin treatment, and the effect of ACTH and cortisone remained long when the administration of these hormones was stopped in alloxan diabetic dog, suggesting the enhanced production of contrainsulin in the endocrine system in alloxan diabetic dog.

REFERENCES

Wakabayashi, K. Preparing for publication