BLOOD SUGAR AFTER INJECTION OF ACETOACETATE

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A number of investigators (1) have attempted to explain the decreased utilization of carbohydrate in conditions accompanying food intakes low in carbohydrate and high in fat. In the case of the formation of increased amounts of ketone bodies due to such conditions or to diabetes, Nath and Brahmachari (2) have attributed at least a part of the decreased utilization of carbohydrate to an inactivation of available insulin by these intermediary fat metabolism products. "Insulin-refractory" cases were explained in this way. They also reported (3) that repeated injections of acetoacetic, \( \beta \)-hydroxybutyric, and pyruvic acids caused hyperglycemia in rabbits, with fasting values increasing from day to day after daily injections.

We had previously noted (4, 5) that rats which had been on a low protein-high fat diet were much more susceptible to ketosis when fasted and that they also exhibited a decreased glucose tolerance. In view of the above findings, it seemed desirable to obtain additional information regarding the effect of the ketone bodies on carbohydrate metabolism in such animals. When rats instead of rabbits were used as the experimental animal, we failed to confirm the findings of Nath and Brahmachari. Instead of elevated blood sugar levels, hypoglycemia developed after the injection of very large amounts of acetoacetate.

EXPERIMENTAL

In the first experiment, female white rats averaging 250 gm. in weight were used to test the hyperglycemic effect of the injection of ketone bodies. Females were selected, since it is possible that ketosis may produce a more marked effect on their carbohydrate metabolism than on that of males (6). All animals of this study were fed a diet similar in composition to that previously used (4), with 25 per cent casein and 15 per cent Crisco. The food was available for 24 hours to a part of the rats, to others for 7 hours, and to still others only 3 hours a day during the preliminary period of 2 weeks for establishing new feeding habits. The animals on each of these feeding régimes were divided into three groups. For blood sugar determinations, 0.2 cc. of blood was milked from the clipped tails of all these animals after 18 hours fasting and at intervals of 1, 2, and 3 hours thereafter. After the fasting blood sample was taken, the first group was injected intraperitoneally with 1 cc. of 1 per cent saline per 100 gm. of body weight the 1st
day, and then with 18 mg. of acetoacetic acid, in 1 cc. of saline solution adjusted to pH 7.3, per 100 gm. of body weight for the next 3 days. The second group received only the saline on all 4 days and the third group was not injected. Four samples of blood for sugar determinations were taken from each animal. All saline injections contained amounts of sodium equivalent to that in the acetoacetic acid.

A second experiment was undertaken to study the effect of injected acetoacetate upon endogenous insulin available for sugar utilization during glucose tolerance tests. Female rats averaging 220 gm. were used for the tolerance tests. Control values were obtained 1 week with glucose in saline and then with glucose in acetoacetic acid the following week. The first four animals received intraperitoneally 3.5 gm. of glucose per kilo in 1 per cent saline, followed by 18 mg. of acetoacetic acid and the same amount of glucose per 100 gm. of body weight. The second group was treated in like manner, except that less glucose, 2.0 gm. per kilo, and twice as much acetoacetic acid were given them.

A study to determine the effect upon the insulin-secreting mechanism of daily injections of acetoacetate in increasing amounts for an extended period was made in a third experiment. The same diet was fed to male rats averaging 230 gm. in weight. Blood sugar determinations were made as controls on the fasting levels and at 1 hour after the subcutaneous injection of 1 per cent saline at the start of the experiment. Similar determinations were made once each week, before and after the subcutaneous injection of acetoacetic acid or saline daily for 4 weeks. The initial injection of 18 mg. of acetoacetic acid per 100 gm. of body weight was doubled each week up to 144 mg. the last week. The controls received saline containing equivalent amounts of sodium. For comparison, the latter amount of acetoacetic acid or saline was injected for only 1 day in other animals (approximately 300 gm.) and blood sugar determined during fasting and 1 hour after injection.

All blood sugars were determined in triplicate by the method described by Nelson (7). The acetoacetic acid injected was prepared by hydrolyzing ethyl acetoacetate with sodium hydroxide in the cold and adjusting finally to pH 7.3 with hydrochloric acid. Urine was collected for 6 hours and then the following 18 hours after all acetoacetate injections, and qualitative tests for ketone bodies were made by the nitroprusside method. The room temperature during these experiments was maintained at 26° ± 1°.

Apparent differences in results were tested for significance by the $t$ method of Fisher (8), and only those having a $P$ value of 0.01 or less were considered significant.

**Results**

The various feeding régimes of the preliminary period in the first experiment did not cause any apparent differences in the blood sugar levels of the
animals which had been divided into the three groups. Hence the data on all of the animals in each of these groups have been pooled. In all cases the initial concentration of the fasting blood sugar on the 3rd day after the acetoacetic acid injections were begun was significantly higher than the control values of the 1st day, as previously reported (3) for rabbits. This was also true for the average values, shown in Table I, on rats whether they received the keto acid, saline only, or no injections whatsoever.

The acetoacetic acid injections did not significantly affect the blood sugar levels during the 3 hours following injection, although ketonuria of varying

**Table I**

*Effect of Injection of Keto Acid and Treatment upon Blood Sugar Levels*

The animals were fasted 18 hours. They received the diet *ad libitum* during the remaining 3 hours after collection of blood samples each day. 1 cc. of 1 per cent saline or 18 mg. of acetoacetic acid per 100 gm. of body weight were injected after collection of the fasting blood sample.

<table>
<thead>
<tr>
<th>Day</th>
<th>No. of rats</th>
<th>Injection</th>
<th>Blood sugar*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fasting</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>Saline</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Keto acid</td>
<td>86 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Saline</td>
<td>93 ± 5</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>Keto acid</td>
<td>92 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>Saline</td>
<td>97 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>Keto acid</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>Saline</td>
<td>100 ± 2</td>
</tr>
</tbody>
</table>

* ± the standard error.

degrees always ensued. The injection of 18 mg. of acetoacetic acid per 100 gm. of body weight was used because it did produce a definite ketonuria and, on a weight basis, was about twice as much as Nath and Brahmachari gave to their rabbits (2).

In the second experiment, the glucose tolerance was not significantly changed from the control values by the simultaneous injection of glucose and acetoacetic acid. A loss of some sugar in the urine and no appreciable change in the tolerance curves in the first animals suggested the use of less glucose and more acetoacetic acid. Both tests, summarized in Table II, gave similar results. There was no indication that the injected ketone body had affected the endogenous insulin available for sugar utilization during these tests.
The results of the last experiment are given in Table III. The daily injection of from 18 to 70 mg. of acetoacetic acid per 100 gm. of body weight during a 3 week period did not significantly change the blood sugar during fasting or 1 hour after injection. Subcutaneous injection was used to prolong the period of absorption. After the injection of 144 mg. of the keto acid each day during the 4th week, a definite hypoglycemia did develop 1

### Table II

**Effect of Keto Acid Injection on Glucose Tolerance**

The four animals in each group were fasted 16 hours. The glucose was dissolved in saline so that the amount injected would contain sodium equivalent to that in the acetoacetic acid solution (pH 7.3).

<table>
<thead>
<tr>
<th>Injection</th>
<th>Blood Sugar*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Acetoacetic acid</td>
</tr>
<tr>
<td>gm. per kg.</td>
<td>mg. per 100 gm. sol.</td>
</tr>
<tr>
<td>3.5</td>
<td>0</td>
</tr>
<tr>
<td>3.5</td>
<td>18</td>
</tr>
<tr>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>2.0</td>
<td>36</td>
</tr>
</tbody>
</table>

* ± the standard error.
hour after the injection. However, in another group of animals, a similar hypoglycemia occurred after only one injection of the latter amount of the keto acid. This suggests that the quantity of the acid given was responsible for the lowered blood sugar and not the repeated daily injections. This quantity appears to be between 70 and 140 mg. per 100 gm. when injected subcutaneously into the rat.

**DISCUSSION**

The effect of the intermediary fat degradation products, the ketone bodies, upon the secreting pancreatic cells and upon the insulin itself would appear to be of considerable importance in carbohydrate metabolism if the views of Nath and Brahmachari are correct. They presented evidence to show that the ketone bodies inactivated insulin, both *in vitro* and *in vivo*, and suggested that the keto acids might first stimulate the pancreatic cells and later cause lesions after fatigue through excessive work.

It seemed possible that the hyperglycemic effect they obtained after repeated injections might be due to the use of a herbivorous animal, or to the short feeding period each day necessary for an 18 hour fast, since a marked change in the intermediary carbohydrate metabolism has been shown to occur after altered dietary habits (9). Also a limited intake of carbohydrate might explain its decreased utilization. In our study neither the injected keto acid nor the short feeding periods can account for the resulting hyperglycemia in the rat. It occurred in the uninjected animal after repeated daily sugar determinations, and normal values were obtained on the 1st day after some time on the 3 hour feeding régime. In contrast, quite similar blood sugar values were obtained when the determinations were made 1 week apart on the animal injected repeatedly (Table III).

The failure of the acetoacetic acid to alter the glucose tolerance and the failure of its repeated injection over a 4 week interval to produce a hyperglycemia do not appear to be compatible with an *in vitro* inactivation of endogenous insulin. However, the hypoglycemia after injection of very large amounts of the keto acid might be explained by a stimulation of the secreting pancreatic tissue. Another interesting possibility is that of a sugar-sparing action of the ketone bodies, associated with decreased glycogenolysis and lowered blood sugar level, since it has been shown that the rate of utilization of the ketone bodies is governed by their concentration in the blood (10) and that they are preferentially used in the presence of usable glucose (11). Also glycogen has been found to be stored in the heart in the presence of large amounts of administered ketone bodies. 1 This study will be continued.

1 Lackey, R. W., personal communication.
A daily hyperglycemia was found in the rat when injected with aceto-acetic acid for 3 consecutive days. The elevated blood sugar apparently was not caused by the ketone body, since the same results were obtained with saline or with no injections. Also blood sugar, before and after the administration of smaller amounts of the keto acid which had been given daily for 3 weeks, was normal if blood samples were taken only 1 day each week. The glucose tolerance was also unchanged by simultaneous injection of the ketone body. However, the administration of a large amount of aceto-acetic acid (140 mg. per 100 gm.) caused hypoglycemia even after a single injection.

Our data do not support the view that insulin is inactivated by the keto acid. The hypoglycemia, after large amounts of the acetoacetate, is compatible with a stimulation of the secreting pancreatic tissue, or a glucose-sparing action associated with a decreased glycogenolysis and hence a lowered blood sugar level.

BIBLIOGRAPHY
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