THE ADRENALS AND THE MOBILIZATION OF STORED FAT FORMED FROM DIETS CONTAINING DIFFERENT FATS

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It has been amply demonstrated that the metabolism of the rat is disturbed by adrenalectomy. The more rapid disappearance of carbohydrate on fasting has been attributed to decreased glycogenogenesis from protein (1, 2). However, the accumulation of fat in the liver is also decreased and is not restored to normal by salts alone (3, 4). Since a large part of the normal metabolism of the animal during fasting is that of fat, it was thought worth while to investigate the rate at which fat disappeared from the liver, mesenteric stores, and skeletal muscle of adrenalectomized rats when food was withheld.

Since tung oil contains a high amount of conjugated fatty acids, one diet contained this fat in an emulsified form, the original plan being to use this as a tracer. This did not prove feasible, but it was found that the presence of this fat considerably increased the excretion of acetone bodies and the loss of fat from the liver during fasting.

Methods

Young male rats of the Sprague-Dawley strain, 75 to 90 days old, were divided into twelve groups of five to eight rats each with similar distributions of body weight. They were then fed one of the two diets listed in Table I for 6 days by the stomach tube method of Reinecke et al. (5).

The sodium bicarbonate was included to increase the ketogenic effect of the diet. Each rat was given 1 cc. of diet twice a day for every 60 sq. cm. of body surface. All animals received the Rubin-Krick solution to drink (6). Two groups were adrenalectomized at the beginning of the feeding period, the lumbar approach being used, and four other groups at the end of the 6 day feeding period. Table II gives an outline of the treatment for each group.

Groups 1, 2, 5, and 6 were placed in metabolism cages during the feeding

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period. The urine was collected in 48 hour portions and analyzed for total acetone bodies by the method of Van Slyke (7). During the fasting period the urine of Groups 11 and 12 was collected and analyzed in the same manner. All values for total acetone bodies are expressed as the equivalent weight of acetone.

The animals were killed at the time indicated in Table II, and the livers were digested with 6 M KOH solution and the fatty acids extracted, titrated, and weighed as previously described (8). In Groups 5 to 12 the perirenal,
pararenal, and gonadal fat depots were also dissected out, dried with anhydrous Na$_2$SO$_4$, ground, and extracted with petroleum ether. Aliquots of the petroleum ether extracts were then dried and weighed. The muscles of the thighs were also removed in these groups, dissected free of observable fat, and treated in the same manner as the fat depots.

Whenever adrenalectomy had been performed, the region around the upper pole of the kidney was examined for adrenal tissue. If any tissue resembled cortical tissue, the area was removed and examined under a dissecting microscope. In two cases tissue of possible cortical character was observed and these rats were discarded. Data for all other rats are included except when samples were lost during analysis.

**Results**

The excretion of acetone bodies is indicated in Fig. 1. The ketosis was relatively low on the diet in which the only source of fat other than the vitamin concentrates was butter fat, even though sodium bicarbonate was administered. The effect of the presence of tung oil as 13.7 per cent of the total fat was quite marked. There was a 3-fold greater excretion of acetone bodies.

Fig. 1 also illustrates the effect of adrenalectomy, and of tung oil in the diet, on the ketosis during a subsequent period of fasting. The adrenalectomized rats excreted only about one-third of the acetone bodies excreted by the controls. They showed a similar curve of excretion during fasting, however. There was a rise during the 3rd and 4th days and a subsequent drop, but not to the level of the first 2 days of fasting.
MOBILIZATION OF STORED FAT

Fig. 2. Effect of fasting on the amount of liver, pararenal and gonadal depot, and muscle fat in normal and adrenalectomized male rats. The bars represent the mean values; the dots represent individual analyses. The value marked with an asterisk was not used in computing the mean.

TABLE III
Fat Content of Organs of Rats with and without Adrenals during Fasting Period

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Time fasted</th>
<th>Fat in diet</th>
<th>Liver fat per 100 gm. body weight</th>
<th>Depot fat per 100 gm. body weight</th>
<th>Muscle fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>mg.</td>
<td>Average and standard error</td>
<td>mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>1. Original</td>
<td>0</td>
<td>Butter</td>
<td>6.75 ± 0.065</td>
<td>5.26 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>2. &quot;</td>
<td>0</td>
<td>Tung oil</td>
<td>5.86 ± 0.024</td>
<td>3.15 ± 0.08</td>
<td>6.1 ± 0.67</td>
</tr>
<tr>
<td>3. Controls</td>
<td>2</td>
<td>&quot;</td>
<td>5.27 ± 0.043</td>
<td>2.26 ± 0.08</td>
<td>5.6 ± 0.47</td>
</tr>
<tr>
<td>4. Adrenalectomized</td>
<td>2</td>
<td>&quot;</td>
<td>5.28 ± 0.043</td>
<td>2.33 ± 0.14</td>
<td>4.2 ± 0.22</td>
</tr>
<tr>
<td>5. Controls</td>
<td>4</td>
<td>&quot;</td>
<td>5.16 ± 0.024</td>
<td>1.74 ± 0.15</td>
<td>3.7 ± 0.30</td>
</tr>
<tr>
<td>6. Adrenalectomized</td>
<td>4</td>
<td>&quot;</td>
<td>4.165 ± 0.013</td>
<td>2.55 ± 0.23</td>
<td>4.5 ± 0.48</td>
</tr>
<tr>
<td>7. Controls</td>
<td>6</td>
<td>&quot;</td>
<td>4.116 ± 0.029</td>
<td>0.99 ± 0.17</td>
<td>2.7 ± 0.14</td>
</tr>
<tr>
<td>8. Adrenalectomized</td>
<td>6</td>
<td>&quot;</td>
<td>4.115 ± 0.015</td>
<td>1.88 ± 0.07</td>
<td>4.4 ± 0.54</td>
</tr>
<tr>
<td>9. Controls</td>
<td>6</td>
<td>Butter</td>
<td>6.35 ± 0.063</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Adrenalectomized</td>
<td>6</td>
<td>&quot;</td>
<td>7.17 ± 0.024</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The effect of the presence of the tung oil in the diet persisted during the subsequent fasting period. Its marked tendency to increase the degree of ketosis was observable in the absence of the adrenals, and was therefore not
mediated through them. The period of highest excretion was not significantly prolonged, however; it was reached on the 3rd and 4th day and was followed by a drop in the subsequent 2 day period.

Fig. 2 and Table III show the change in fat content of the liver, fat depots, and muscles both after adrenalectomy and on the two types of fat diet. The removal of the adrenals did not significantly change the rate at which the fat content of the liver fell during fasting when the animal had previously been on the tung oil diet. When the previous diet had contained only butter fat, however, the decrease in liver fat was less in the controls than in the groups on tung oil. The difference was highly significant ($P = <0.01$ for Groups 7 and 9 and Groups 8 and 9). Removal of the adrenals appeared to have accelerated the decrease until it was approximately the same as in the animals fed tung oil. The probability that the difference between the means for the control and adrenalectomized rats on the butter fat diet was due to chance was 0.02.

Removal of the adrenals did affect the rate at which fat disappeared from the fat depots and muscles, however. The change during the first 2 days after the operation was similar in both groups, probably because of the effect of circulating hormone. After this, however, the decrease was slow and irregular in the adrenalectomized group, while the controls continued to use fat at a steady rate. The net utilization of stored fat during fasting seemed to be accelerated by the presence of the adrenal glands. The differences by the 6th day were highly significant: for muscle fat $P = 0.02$ and for depot fat $P = <0.01$.

During the feeding period, adrenalectomized rats accumulated only half as much fat in the livers as did the controls. This was true on either the tung oil or butter fat diets.

**DISCUSSION**

It would seem that the results obtained in this study could be best explained by two assumptions: that adrenalectomy decreased the rate of transport of fat from the body stores to the liver or its uptake by the liver, and that the presence of tung oil increased the rate of fat metabolism in the liver.

The lipolytic action of the tung oil would explain the increased ketosis in the normal animals on this diet and in subsequent fasting, associated with a more rapid drop in both liver and depot fat while fasting.

On this basis the ketosis was reduced in the adrenalectomized rats, because the fat was not removed by the liver with sufficient rapidity to enable it to form acetone bodies at the normal rate. This is borne out by the slower fall in depot fat during fasting. The lower level of liver fat during feeding with either diet and the more rapid decrease in liver fat
MOBILIZATION OF STORED FAT

during fasting in the adrenalectomized rats fed butter fat are also compatible with this view. Barnes et al. (9) have shown that conjugated fatty acids in the neutral fat fed did not accumulate as rapidly in the livers of adrenalectomized rats as in the normals, although they appeared with normal rapidity in the phospholipid fraction. In cats, Yeakel and Blanchard (10) report lower plasma lipids after adrenalectomy. This would agree with the general thesis that adrenalectomy interferes with the passage of neutral fats (e.g. depot fat) into the hepatic cells and thus lowers the fat available for metabolism by the liver.

If this is true, however, why did not the liver fat in the adrenalectomized rats on the tung oil diet fall more rapidly than that of the controls during fasting? One explanation would be that the fats were being metabolized so rapidly after the tung oil diet that the rate of fall of the liver fat was maximal. When one considers that the fat content of the livers fell from an average of 0.9 gm. per 100 gm. of body weight to 0.1 gm. per 100 gm. of body weight in a period of 6 days during which fat mobilization and utilization was rapid, it is obvious that fat was passing out of the liver at a high rate. This is confirmed by the average acetone excretion of 71.5 mg. per day for a 150 gm. rat. In the adrenalectomized rats previously on the butter fat diet this rate of disappearance was approached but was not quite reached. If, then, this represents the maximal rate of fat disappearance under the circumstances, it is understandable that the adrenalectomized rats did not lose fat from the livers at any greater rate. The difference due to adrenalectomy should be reflected, rather, in a greater difference in acetone body excretion. This appears to be the case; the excretion in the animals without adrenals is one-third that of the controls when both have previously been on a butter fat diet, while it is only about one-fourth that of the animals on the tung oil.

Verzar and Laszt (11) and Bavetta and Deuel (12) have introduced evidence favoring a delayed absorption of fatty acids as an important factor in fat metabolism of the adrenalectomized rat. While there may have been a small decrease in absorption, it is doubtful whether this could be a significant factor in the differences in liver fat and acetone body excretion, since the differences were similar during feeding and when adrenalectomy was performed after the last food was fed; in the latter case absorption could not possibly be the causal factor.

SUMMARY

Male rats 75 to 90 days of age were fed for 6 days on high fat diets, containing either almost all butter fat or 17 per cent tung oil and 73 per cent butter fat. They were then fasted for 6 days. Some rats were adrenalectomized at the beginning of feeding and some at the start of the fasting
period. Groups of both adrenalectomized and control rats were killed at the end of feeding and at intervals during the fasting period.

The presence of tung oil in the diet increased the acetone body excretion in all animals both during feeding and in the subsequent fasting period.

On fasting the ketosis increased, reaching a peak on the 3rd and 4th days.

The ketosis was greater when the rats had been on a tung oil diet if normal or adrenalectomized rats were compared with similar rats fed butter fat only.

The acetone body excretion was always greater in the normal fasting rats than in adrenalectomized rats on the same diet.

The liver fats of both adrenalectomized and control rats on the tung oil diet fell rapidly during fasting. The adrenalectomized rats used up their depot fats at a much slower rate than the controls, however. The liver fats of control rats which had been on a straight butter fat diet did not decrease as rapidly on fasting as in the adrenalectomized rats or the rats previously on the tung oil diet.

An explanation of the data is offered on the assumption that the tung oil primarily affected the breakdown of fat in, and transport from, the liver, while adrenalectomy primarily affected the transport from the depots to the liver.

Since the same type of differences appeared in fasting between controls and rats adrenalectomized after the last feeding as occurred between the two types of rats during feeding, it seems unlikely that changes in fat absorption were major factors in the differences in liver fat and acetone body excretion.

BIBLIOGRAPHY

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