The rapid onset of a marked fasting ketosis following low protein diets has been attributed by MacKay et al. (1, 2) to a lack of "stored" protein. They suggested that the catabolism of the "stored" protein after high protein diets, as measured by the urinary nitrogen excretion, supplied sufficient antiketogenic material (glucose) to reduce the need for metabolizing fats. Besides the increased excretion of nitrogen in the urine, the better maintenance of the blood sugar and the liver glycogen levels in fasting rats previously receiving more protein was cited as additional evidence for this mechanism.

Although there is considerable evidence (2, 3) that the protein of the preceding diet is related to the following fasting ketosis, it seemed desirable to obtain further data before concluding that the antiketogenic material from the protein catabolized during fasting, as measured by the nitrogen excreted, is sufficient to account for the low level of ketonemia following the higher protein diet. Also, a part of the small increase in nitrogen excreted by animals which had been on the 25 per cent protein diet, as compared with that of the animals which previously received a 5 per cent protein diet, might be due to either the well known lag in the excretion of the nitrogenous end-products of protein metabolism or to an excessive flushing out of these products from the body by the diuresis which was produced as a part of the experimental procedure (2).

The present report discusses the results of experiments designed to give additional information concerning two points in relation to fasting ketosis. First, is the extra nitrogen excreted by fasting animals previously on high protein diets (in comparison with those receiving low protein diets) an accurate measure of the quantity of antiketogenic material available from the "stored" protein catabolized in these animals? Secondly, does the "stored" protein catabolized during fasting supply sufficient antiketogenic material to prevent the development of a fasting ketonemia comparable to that observed in animals which have received low protein diets? In regard to the first point, this study indicates that the extra nitrogen excretion is a relatively accurate measure of the "stored" protein catabolized.

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Concerning the second, our results show that the administration of glucose, equivalent to the carbohydrate made available by catabolism of the extra protein (as calculated from the nitrogen excretion), did not produce in rats previously on a low protein diet the low level of fasting ketonemia observed in animals which had received high protein diets.

**EXPERIMENTAL**

In the first experiment white male rats averaging 200 gm. in weight were fed Diets 1 and 2 for 18 days. Diet 1 contained 5 per cent casein and 34 per cent each of starch and glucose; Diet 2, 25 per cent casein and 24 per cent each of starch and glucose. Both diets contained 2 per cent Cellu flour, 5 per cent salt mixture (4), and 20 per cent lard. The diets were supplemented daily with 1 dried yeast tablet and 2 drops of cod liver oil. Beginning with the 19th day, all animals were fed Diet 1 for the next 3 days and then fasted for 2 days. The low protein diet was given for 3 days before the fast to the animals previously receiving 25 per cent protein to avoid any lag in the excretion of the nitrogenous end-products while fasting as a result of the earlier higher protein intake. Hence, the nitrogen excreted during the fast should be a truer measure of the protein catabolized. Diet 1 served as a maintenance diet for these animals, since all maintained their weight or gained slightly during the 3 day period. The daily food intake and weight changes were recorded. Daily urine collections were made under light mineral oil during the last 8 days of the study and the urine was analyzed for nitrogen. The adequate urine volumes previously found necessary for nitrogen excretion studies (2) were obtained by the intraperitoneal injections twice daily of 5 cc. of 0.9 per cent saline. The saline solution was administered for 6 days before the fast to prevent the flushing out of the end-products of protein metabolism during the fast, which might have occurred if the solution had been given only during the fasting period. Total blood ketone bodies were determined at the end of the 1st and 2nd days of fasting, as previously described (3).

In the second experiment, similar rats weighing approximately 225 gm. were fed Diet 3 for 21 days. This diet was similar to Diet 1 with the exception that the carbohydrate and fat contents were changed to 48 per cent glucose and 40 per cent lard. All of the animals received approximately the same amount of food, by limiting the food to that amount the majority would eat, in order that the body stores would be as nearly the same as possible when they were later divided into two groups. To determine more accurately the start of the fasting periods, the food cups were removed from the cages at 8 a.m. and returned at 9 a.m. On the day the fasting periods began, the cups were not returned to the cages and 9 a.m. was considered

1 Generously supplied by the Corn Products Refining Company, New York.
Intraperitoneal injections of 0.3 cc. per sq. dm. of body surface, calculated according to the formula of Rubner (5), of 0.9 per cent saline or 5 per cent glucose were given at the start of the fast and at the end of each 12 hour period of the 48 hour fast. This 15 mg. of glucose per unit of body surface given twice daily is equivalent to the antiketogenic material available from the metabolism of protein containing 8 mg. of nitrogen, assuming that the protein is 16 per cent nitrogen and that 58 per cent of the protein is available for antiketogenic action. The blood for ketone determinations was drawn just before the injections at the end of 24 and 48 hour fasting periods. The room temperature during both experiments was maintained between 25-27°. Apparent differences were tested for significance by the t method of Fisher (6), and only those having a P value of 0.01 or less were considered significant. The standard error of the mean is given with the tabulated data.

Results

The nitrogen excretion of the animals on the higher protein diet promptly decreased when the animals were placed on the 5 per cent protein diet. As is shown in Table I, the average nitrogen excretion for this period was approximately the same as that of the 2 day fast, indicating that any lag in the excretion of nitrogen had been eliminated. However, there was still a significant difference in the nitrogen excreted during the fasting period by the two groups previously on the diets of differing protein content. An excess of approximately 8 mg. of nitrogen per unit of body surface per day was excreted by the animals previously on Diet 2. The shift from the high to a low protein diet for 3 days and the prolonged diuresis had not altered the significant difference in ketonemia after a 48 hour fast following the low and high protein diets.

Additional evidence regarding the nitrogen excretion during a 2 day fast was obtained by calculation with the data on the absolute weights of liver and other body proteins of animals from a previous experiment (7). The animals were the same strain and sex as those used in this study. Their weight, environment, and diets were quite comparable to those of the animals from which the nitrogen excretion data were obtained. The amount of protein available for catabolism was calculated according to Addis et al. (8), who reported losses of 20 per cent liver protein and 4 per cent of other body proteins in rats during a 2 day fast. In Table II are shown the differences between the liver and body proteins of the unfasted animals previously on low and high protein diets and the calculated losses during a 2 day fast. The excess protein calculated to be lost during the 2 day fast contained approximately 7 mg. of nitrogen per unit of body surface. This value is slightly less than the daily nitrogen excretion value of 8 mg. that we obtained experimentally.
The effect of injecting 15 mg. of glucose per sq. dm. of body surface twice daily on fasting ketonemia after the low protein diet is shown in Table III. This glucose, the equivalent of the antiketogenic material from the excess protein catabolized during fasting following the high protein diet, was given to determine whether the effect on the following fasting ketonemia would be comparable to that of a preceding high protein intake. The level of

### Table I

**Nitrogen Excretion and Fasting Ketonemia**

The rats were fed Diets 1 and 2 for the first 18 days. Then all were fed the 5 per cent protein diet during the 19th and 21st days and were fasted the 22nd and 23rd days. 24 hour urine collections were made for nitrogen determinations.

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>No. of rats</th>
<th>Initial weight</th>
<th>Changes in weight</th>
<th>Protein intake per 100 gm. rat per day</th>
<th>Nitrogen excretion, sq. dm. per day</th>
<th>Fasting ketonemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm. per cent</td>
<td>gm.</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>203</td>
<td>9.2</td>
<td>0.73</td>
<td>±0.8</td>
<td>9.7</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>200</td>
<td>19.4</td>
<td>2.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>±0.7</td>
<td>±0.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> During first 18 days; 2.62 gm. for 21 days.

### Table II

**Calculated Protein Available During 2 Day Fast**

The data including absolute weights of protein were obtained from a previous study (7). The excess available protein in rats on the 20 per cent protein diet over that of those fed the 5 per cent protein diet was calculated on the basis of a loss of 20 per cent of liver protein and 4 per cent of other body proteins during a 2 day fast (8).

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Diet protein</th>
<th>Body surface</th>
<th>Absolute weights of protein</th>
<th>Protein per sq. dm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>gm.</td>
<td>gm.</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>2.43</td>
<td>1.050</td>
<td>22.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.05</td>
<td>±0.048</td>
<td>±0.79</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>2.87</td>
<td>1.281</td>
<td>29.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.03</td>
<td>±0.036</td>
<td>±0.73</td>
</tr>
</tbody>
</table>

Differences

Calculated protein available during fast

Nitrogen from available protein

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Body</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td>gm.</td>
</tr>
<tr>
<td>0.231</td>
<td>7.20</td>
<td>0.014</td>
</tr>
<tr>
<td>0.003</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>0.000</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>
the blood ketone bodies appeared to be slightly lowered by even this small amount of glucose, but the difference between the ketone levels of these animals and the saline-injected controls was not statistically significant. Also, the ketonemia after the glucose injections was similar to the 26.9 mg. per cent value obtained in the first experiment after the 5 per cent protein diet when neither glucose nor saline was given during the fast.

The blood ketone values observed in this study were lower than some previously reported by this laboratory (3). These animals, which were obtained from a different commercial source, appeared to be more resistant to ketosis than those of the earlier studies. The higher values reported by others (2) may also be explained by the fact that their fasting periods began 15 hours after the removal of the food instead of at that time.

Table III

Effect of Saline and Glucose Injections on Fasting Ketonemia

In two groups, each containing twelve animals, the food intake per day was 7.1 gm. All the animals received a 5 per cent protein diet (No. 3) for 21 days. Injections of 0.3 cc. per sq. dm. of body surface of 0.9 per cent saline or 5 per cent glucose were given twice daily during the following 48 hour fast. This amount of glucose is that available from the metabolism of protein containing 8 mg. of nitrogen.

<table>
<thead>
<tr>
<th>Body weight gm.</th>
<th>Change in weight per cent</th>
<th>Solution injected</th>
<th>24th hr. mg. per cent</th>
<th>48th hr. mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>223</td>
<td>-14.8</td>
<td>Saline</td>
<td>15.2 ± 1.0</td>
<td>28.5 ± 1.0</td>
</tr>
<tr>
<td>225</td>
<td>-14.2</td>
<td>Glucose</td>
<td>11.2 ± 0.6</td>
<td>25.6 ± 1.3</td>
</tr>
</tbody>
</table>

Discussion

An excess of 8 mg. of nitrogen per sq. dm. of body surface per day was excreted during the 2 day fast by the animal previously on the higher protein intake, even after a preceding protein maintenance diet for 3 days and 6 days of diuresis. It appears that this persisting difference in nitrogen excretion following the low and high protein diets cannot be ascribed to a lag in nitrogen excretion or to an excessive flushing out of additional nitrogenous end-products following the higher protein intake. Rather, the difference may be attributed to the catabolism of more protein in the fasting animal following the higher protein diets. This difference in excretion of 8 mg. of nitrogen agrees closely with the approximately 6 mg. value obtained by MacKay et al. (2) under similar experimental conditions with the exception of the prefasting maintenance diet and a shorter period of diuresis.

It appears significant that the calculated excess protein lost by the fast-
ing animals after the higher protein diet (Table II) should account for only slightly less nitrogen than the experimental values obtained in this study and in that of MacKay and coworkers. The evidence suggests that this amount of nitrogen (4 to 8 mg. per sq. dm. per day) is a measure of the excess protein catabolized by the fasting animal after higher protein intakes and that the higher value would represent the maximum amount of protein that might be catabolized to supply excess antiketogenic materials to these animals.

When the absolute amounts of liver and the remaining body proteins were converted to amounts per unit of body surface, the amount of liver protein was found to be approximately the same in the animals on both low and higher protein diets. A significantly greater amount of body protein occurred in the animals on the higher protein intake. This suggests that, under the conditions of this study, the body stores and not those of the liver supplied the additional protein metabolized in these animals with larger stores.

A supplement of glucose, equivalent to the excess protein catabolized during fasting after the higher protein diets, failed to produce the same low level of fasting ketonemia as did a previous higher protein intake. There appeared to be a slightly lower level of ketonemia in the fasting animals receiving this small amount of glucose, but the level was significantly higher than that of the animals previously on the higher protein intakes and was not significantly different from that of the animals after the low protein diets. Thus the available carbohydrate from the additional catabolized protein appears not to be the major factor responsible for the lower level of fasting ketosis after high protein diets, and this effect must at least in part be otherwise explained.

Evidence is being accumulated that suggests an increased rate of utilization of carbohydrate by the animals on the low protein diets, when fed and extending into the early fasting period. Other investigators (9, 10) have suggested alterations in the carbohydrate metabolism as a result of dietary changes. The rapid disappearance of liver glycogen during early fast (11) could be due to a more rapid utilization as well as a lack of replacement by glyconeogenesis. On a similar caloric intake, animals on the low protein diets usually lose weight or barely maintain it, while those on higher protein intakes make appreciable gains in weight. This suggests an increased rate of metabolism in these fed animals on low protein diets which would account for the early ketosis following the rapid loss of carbohydrate stores. This metabolic rate apparently decreases to below the normal levels after an overnight fast, since several investigators (12, 13) have shown a lower than normal basal rate in these animals. Further studies are in progress.
SUMMARY

The increased nitrogen excretion during fasting after the higher protein diet was not eliminated by 3 days on a 5 per cent protein diet and 6 days of diuresis preceding the fast. Hence it appears not to be due to a nitrogen lag or an excessive flushing out of the metabolic end-products during the fast. A similar nitrogen value is accounted for by the extra protein calculated as lost from the animals during the fast. This excess nitrogen excreted thus appears to be due to the additional protein catabolized in these animals.

A glucose supplement equivalent to the amount that would be available from the excess protein catabolized after the higher protein diet failed to produce the antiketogenic effect previously attributed to it. Thus the amount of protein available for catabolism during fasting does not appear to be the major factor responsible for the degree of fasting ketosis.

The greater fasting ketosis after a low protein intake may be related to an increased utilization of carbohydrate in these animals. This change in metabolism is suggested by the rapid disappearance of liver glycogen during the early fast and by body stores smaller than those of animals following high protein diets after similar caloric intakes.

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