THE RELATION OF FASTING KETOSIS IN THE RAT TO THE PRECEDING DIET AND THE LIVER FAT

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When rats are fasted they develop a ketonemia (ketosis) and frequently it is high enough to produce a measurable ketonuria. The height of the ketosis is determined by many factors. When the diet is low in choline and in protein, the liver becomes very fatty (1) and the ketonuria on subsequent fasting is much higher (2, 3) than it is in animals that have been on the stock diet or other food mixtures with a higher protein content. It seemed reasonable to assume that the high degree of ketosis during fasting after a low protein intake was incident to the fatty liver. This appeared probable, for ketone bodies arise from the oxidation of fatty acids in the liver, and, during fasting after ordinary diets, fat generally accumulates in the liver during the period in which the ketosis is reaching its ultimate level. However, we have been unable to obtain a good correlation between the degree of ketosis and the amount of fat in the liver and have therefore been led to examine some of the dietary factors which determine the extent of the fasting ketosis.

Methods

The experimental methods are the same as those we have used in other studies. Urine specimens were at first collected under light mineral oil (No. 3, Standard Oil Company of California) to prevent loss of acetone. We now prefer this method for collecting rat urine for almost any purpose. The oil prevents evaporation of the small specimens and loss by wetting of containers during measuring and handling.

Analytical methods for blood ketone bodies (4), urine ketone
bodies (5), urine nitrogen, liver glycogen (6), and liver fat (7) were the usual ones. The liver fat as measured by this method comprised the fatty acids of the neutral fat plus the non-saponifiable lipid material. The blood ketone body values are expressed as acetone.

**Influence of Lipotropic Factors**—Under most dietary conditions choline has a powerful lipotropic effect, tending to prevent the accumulation of fat in the liver and driving out hepatic fat which has already been deposited (8). But Deuel *et al.* (9) have found that choline administered during the period of fasting does not appreciably affect the ketosis of fasting rats with fatty livers. We have confirmed this observation (Experiment 1, Table I). Neither does the addition of choline to the diet prior to fasting influence the subsequent ketosis (Experiment 2, Table I), although we know that it prevents any accumulation of fat in the liver.

It has been shown that all or most of the lipotropic activity of

### Table I

**Influence of Choline upon Fasting Ketosis**

**Experiment 1**—Choline was fed during the fasting period. Adult male rats averaging 230 gm. in weight were on Diet 1 (casein 5, Anheuser-Busch's brewers' yeast 5, sucrose 45, Osborne and Mendel's standard salt mixture 5, Crisco 20, butter fat 20) for 10 days and fasted directly from it. Three rats in each group were sacrificed daily for blood ketone determinations. The control group was given 1 cc. of 0.128 N NaCl and the choline-treated group 1 cc. of very nearly neutralized (freshly prepared) 2 per cent choline chloride per sq. dm. of body surface twice daily.

**Experiment 2**—Choline was fed during the period prior to fasting. Adult female rats averaging 203 gm. in weight were on Diet 1 for 10 days. The diet fed the choline group was the same but contained 2 per cent choline hydrochloride. Two rats were sacrificed for the determinations which gave each figure.

<table>
<thead>
<tr>
<th>Time of fasting, hrs.</th>
<th>Blood ketone bodies</th>
<th>Liver fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 mg. per cent</td>
<td>48 mg. per cent</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>35</td>
<td>46</td>
</tr>
<tr>
<td>Choline</td>
<td>49</td>
<td>46</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>Choline</td>
<td>11</td>
<td>32</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
protein is due to methionine (10) and that, while this amino acid keeps fat from the liver, cystine is very active in causing the deposition of liver fat (11). From Experiment 3 (Table II) we see that neither of these substances when added to the diet prior to fasting affects the subsequent fasting ketosis. By the end of the 4th day the blood ketone level was of the same magnitude in every case. It should be noted that the blood ketone level before the 4th day was lower in both of those groups (Experiment 3, Groups 7 and 8) which had been receiving a diet of normal pro-

**Table II**

*Influence of Methionine and Cystine upon Fasting Ketosis*

Experiment 3—Methionine and cystine were fed prior to fasting. Male rats averaging 251 gm. in weight were on the special diets for 10 days. The dl-methionine and l-cystine were added to those diets. There were eight rats on each diet and two were sacrificed for blood ketone determinations at the end of every 24 hours of fasting.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Diet No.</th>
<th>dl-Methionine per cent</th>
<th>l-Cystine per cent</th>
<th>Blood ketone bodies during fasting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hrs. mg. per cent</td>
<td>48 hrs. mg. per cent</td>
<td>72 hrs. mg. per cent</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>28</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
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<td>43</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>18</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>27</td>
<td>37</td>
<td>42</td>
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<td>1</td>
<td>29</td>
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<td>48</td>
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<td>6</td>
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<td>29</td>
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<td>40</td>
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<td>7</td>
<td>2</td>
<td>22</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>25</td>
<td>16</td>
<td>26</td>
</tr>
</tbody>
</table>

* The composition of Diet 1 is given in Table I. Diet 2 was the same as Diet 1, but casein and sucrose were changed to 25 parts each.

Relation of Ketosis to Liver Fat and Glycogen Level—This led us to follow in detail the development of ketosis during fasting following a low protein intake (Experiment 4, Fig. 1). The rats had not been on this diet long enough for their livers to become fatty and the liver fat concentration increased regularly throughout the period of fasting. But the ketosis as measured by the blood ketone level reached its maximum long before the liver fat at-
tained a very high level. The degree of ketosis shows a more probable causal relation to the liver glycogen level. During fasting, the sole source of glucose is from protein catabolism and the glycerol of fat. These rats had been receiving a low protein diet and the amount of "stored" protein available for catabolism must have been very low. This would account for the rapid rise of the blood ketone bodies to their final maximal level. It would seem probable then that the protein content of the preceding diet is the factor chiefly responsible in determining the rapidity with which the maximum ketosis is reached during fasting as well as the level ultimately attained.

*Influence of Protein Intake in Preceding Diet*—To determine whether or not this is the chief factor governing fasting ketosis, rats were fed protein at various levels. In the first experiments, of which a preliminary report has been made (12), choline was
included in the diets, so that even at the lowest protein level fat would be excluded from the liver. The results showed a very definite influence of the prior protein intake on the subsequent fasting ketosis even when this was measured by the ketonuria. A more convincing experiment has been carried out (Experiment 5, Fig. 2), in which the ketosis was measured by the degree of ketonemia. The influence of the preceding diet—the less the protein, the greater the ketosis—is quite striking. In our preliminary report (12) we noted that, “The ketosis then might be dependent upon the antiketogenic action of the amount of ‘stored’ protein now available for catabolism. However, nitrogen excretion figures do not support such a supposition.” This last remark was undoubtedly due to our having available only the nitrogen.

Fig. 2. The development of fasting ketosis in the rat following preceding diets containing variable amounts of protein. Experiment 5, female rats 95 days old and averaging 150 gm. in weight were used. Each observation is an average of determinations made on two rats. The lowest protein diet contained casein 5, sucrose 60, standard salt mixture 5, yeast 5, cod liver oil 5, and Crisco 20. The diets containing larger quantities of protein were similar except that part of the sucrose was replaced by the stated percentage of casein. The animals were on the special diets for 10 days prior to fasting.
figures for the first 2 days of fasting and these had not been obtained with high urine volumes, so that the collection error might be minimized. An experiment was carried out in which careful attention was given to accurate urine collections and with high

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**Fig. 3.** The relation of fasting ketosis, protein catabolism, blood sugar, and liver glycogen to the protein content of the preceding diet. Experiment 6, each point is an average obtained from observations on three rats. Diet Group 1—-, low protein diet; diet Group 2—-, moderate protein diet; diet Group 3—-, high protein diet.
urine volumes, so that the urine nitrogen might more nearly represent the end-products of nitrogenous metabolism during the urine collection periods.

In Experiment 6, male rats 100 days old and averaging 241 gm. in weight were fed for 18 days upon special diets and then fasted. All three of the diets were composed of lard 19, Osborne and Mendel's (13) standard salt mixture 5, Anheuser-Busch's high vitamin brewers' yeast 5, cod liver oil 1, and variable amounts of casein and sucrose. Diet 1 contained casein 5 and sucrose 65. Diets 2 and 3 contained casein 25 and 55 respectively, and correspondingly less sucrose. For diet Groups 1, 2, and 3 the average body weights at the end of the feeding period were 237, 269, and 250 gm., while the average food intakes per rat per day were 8.6, 9.4, and 7.9 gm. respectively. Eighteen rats were fed each diet and three from each diet group were sacrificed at the beginning of the fasting period and at the end of every 24 hours thereafter for 5 days. Each rat was given 5 cc. of water by stomach tube twice daily to insure good urine volumes. The results are summarized in Fig. 3. They show very clearly the dependence of the protein ("stored protein") catabolized during fasting—measured by the urine nitrogen excretion—upon the height of the prior protein intake as varied by the protein content of the previous diet. The ketosis, measured by the level of ketone bodies in the blood, bears an inverse relation to the degree of fasting protein catabolism. This is presumably due to the production of antiketogenic material (i.e. glucose) from the protein, thus reducing the need for ketone bodies. Excellent support for this view comes from the better maintenance during fasting of the blood sugar as well as the liver glycogen level in those rats (Fig. 3, diet Group 3) which had been receiving the most protein prior to fasting and which catabolized the most during fasting. We have noted the higher fasting liver glycogen after a high protein diet before (14).

SUMMARY

Neither the liver fat content per se nor any of the agents such as choline, methionine, or cystine which are known to influence the amount of fat in the liver has a significant effect upon the degree of fasting ketosis in the rat.
Fasting Ketosis

The rapidity of onset and the degree of ketosis reached during fasting bears an inverse relation to the protein content of the preceding diet. This fasting ketosis is apparently related to the protein intake preceding the fasting period because the latter determines the amount of ("stored") protein available for catabolism during fasting. This serves as a source of antiketogenic material and fasting rats, previously on a high protein intake, better maintain their liver glycogen and blood sugar levels as well as have a lower level of blood ketone bodies.

BIBLIOGRAPHY

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