KETOGENIC ACTIVITY OF ACETIC ACID

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It is well known (1-5) that acetic acid is rapidly utilized when fed to mammals. Whether it is oxidized directly or after conversion into other compounds is uncertain. Loeb (6) and Friedman (7) have shown that acetic acid increases the yield of acetoacetate in the perfused isolated liver. The present study was designed to determine whether or not acetic acid is ketogenic in the intact organism.

The macro-Kjeldahl procedure was used for urine nitrogen determinations, Van Slyke's method (8) for the determination of urine acetone bodies, and the method of Barnes and Wick (9) for the determination of blood acetone bodies. Other methods are those which we have used before (10-12) in related studies.

After many unsuccessful trials a satisfactory experiment was carried out in which acetic acid was fed to a phlorhizinized dog. As Deuel and Milhorat (5) have noted, vomiting occurs as a usual thing after the administration of sodium acetate to dogs. Free acetic acid is even worse in this regard and only in this one experiment was emesis avoided. The cathartic action of the salt (5) is not shared by the free acid. The results in Table I show that when acetic acid is fed there is a striking increase in the excretion of acetone bodies in the urine. This was accompanied by a marked rise in the urine nitrogen excretion.

When acetic acid is fed to fasting rats (Table II), there is always an increase in the ketonuria. This is true whether the acetic acid is fed as such or partially or entirely neutralized. Blood acetone body determinations indicate that this increased urinary excretion is not a result of changes in the renal threshold but results from an increased production of acetone bodies. That
the blood acetone body levels of the acetic acid-fed animals are not greatly higher than the controls is due to the fact that the determinations were made 12 hours after the last feeding of the acid. With antiketogenic substances this time element is largely absent in so far as the decrease in the blood ketone level is concerned, for the antiketogenic action is due to the glycogen which is formed and this exerts a fairly constant action over a long period (12).

Acetic acid then is ketogenic in fasting rats as well as in the phlorhizinized dog. It is interesting in this regard that acetic

TABLE I
Influence of Feeding Acetic Acid on Urinary Excretion of Ketone Bodies in Phlorhizinized Dog

A canine bitch weighing 11.6 kilos was fasted for 10 days and observations made the last 6 days. It was given a subcutaneous injection of 10 cc. of 10 per cent phlorhizin suspended in olive oil every day. Urine specimens were collected by catheter. The acetic acid was fed by stomach tube in 3 per cent solution.

<table>
<thead>
<tr>
<th>Length of period</th>
<th>Urine nitrogen (gm. per hr.)</th>
<th>Urine ketones (gm. per hr.)</th>
<th>Acetic acid fed during period (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs.</td>
<td>0.347</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>24 hrs.</td>
<td>0.355</td>
<td>0.060</td>
<td></td>
</tr>
<tr>
<td>20 hrs.</td>
<td>0.663</td>
<td>0.195</td>
<td>10</td>
</tr>
<tr>
<td>24 hrs.</td>
<td>0.784</td>
<td>0.242</td>
<td>20</td>
</tr>
<tr>
<td>25 hrs.</td>
<td>0.490</td>
<td>0.122</td>
<td>10</td>
</tr>
<tr>
<td>15 hrs.</td>
<td>0.408</td>
<td>0.080</td>
<td></td>
</tr>
<tr>
<td>7 hrs.</td>
<td>0.288</td>
<td>0.023</td>
<td></td>
</tr>
</tbody>
</table>

Acetic acid causes a marked increase in the total metabolism (3) and Lusk (13) has observed that, "Just as carbohydrate and fat, when given together, increase the metabolism by the sum of the effects which either alone would produce, so between carbohydrate and acetic acid there was a summation of effect." There is some evidence that acetic acid may be utilized directly by the muscles. However, when acetone body production is taking place, it is probable that exogenous acetic acid is utilized by the organism after conversion to acetone bodies in the same manner as other fatty acids with an even number of carbon atoms. While the higher acids may reach this form by \( \beta \) oxidation, acetic acid must.
### Table II

**Ketogenic Activity of Acetic Acid in Fasting Rats**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>No. of rats</th>
<th>Average body weight</th>
<th>Solution fed in doses of 1 cc. per sq.dm. body surface</th>
<th>Urine N per sq.dm. body surface per day</th>
<th>Urine acetone bodies per sq.dm. body surface per day</th>
<th>Blood acetone bodies at end of day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gm.</td>
<td>sq.dm.</td>
<td>mg.</td>
<td>mg. mg. mg. mg.</td>
<td>mg. per cent mg. per cent mg. per cent</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>173</td>
<td>3.5</td>
<td>1.0 m NaHCO₃</td>
<td>30.5 5.0 3.0 2.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>199</td>
<td>3.9</td>
<td>0.75 m acetic acid</td>
<td>15.7 6.0 16.0 16.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>183</td>
<td>3.7</td>
<td>0.75 m acetic acid + 0.75 m acetic acid</td>
<td>18.3 7.0 12.3 24.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>151</td>
<td>3.2</td>
<td>0.75 m acetic acid</td>
<td>21.5 0.8 2.2 2.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>201</td>
<td>3.9</td>
<td>0.25 m NaCl</td>
<td>19.0 1.3 23.3 17.6</td>
<td>50 67</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>261</td>
<td>4.6</td>
<td>0.25 m acetic acid + 0.25 m acetic acid</td>
<td>16.9 15.3 18.9 13.6</td>
<td>41 47 40 45</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>237</td>
<td>4.3</td>
<td>Water</td>
<td>15.3 12.1 22.7 16.2</td>
<td>58 61 45 44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>237</td>
<td>4.3</td>
<td>0.25 m acetic acid + 0.25 m acetic acid</td>
<td>18.7 22.2 24.1 20.9</td>
<td>61 72 50 59</td>
</tr>
</tbody>
</table>

* Male rats were used in Experiments 1, 6, and 7. Female rats comprised the other experiments.

† Two doses of the solutions listed were fed each day except in Experiments 5 and 6 in which three and four doses respectively were administered daily.

‡ The rats in Experiments 1 and 4 were fasted directly from the stock diet, while all of the rest received a low protein diet for 4 to 23 days prior to fasting.
be converted to acetoacetic acid by condensation. It is known (14) that in the form of its ester acetic acid may be condensed in the test-tube to the ester of acetoacetic acid.

The increase in nitrogen excretion produced in the phlorhizinized dog by feeding acetic acid occurs to a less extent in the fasting rat. It cannot be the result of an acidosis as is the increased protein catabolism produced by feeding inorganic acids, for acetic acid does not reduce the carbon dioxide-combining power of the blood (4). The effect of acetic acid on nitrogen metabolism may not be specific, but a property of other fatty acids as well. Data on this point are not available.

This demonstration that acetic acid may give rise to acetone bodies in the intact mammalian organism has an important bearing upon the current conception of the method by which fatty acids are oxidized in the organism under circumstances such that acetone bodies are being formed. Acetone bodies are produced in the liver and carried by the blood stream to the muscles and other tissues for final oxidation. The old conception of $\beta$ oxidation with the production of only 1 molecule of acetone body from 1 molecule of even the long chain fatty acids is no longer tenable. On this basis an impossible amount of fat would have to be burned to furnish the quantity of acetone bodies which is known to be utilized during a ketosis as a result of studies (15) of the difference in the concentration of acetone bodies in the arterial and venous blood. In a study of the oxidation of fatty acids by isolated liver slices Jowett and Quastel (16) found a larger quantity of acetone bodies than successive $\beta$ oxidation as usually considered would account for. Then Butts (17) and Deuel (18) and their coworkers observed in rat feeding experiments a far greater yield of acetone bodies from the higher fatty acids than Knoop’s hypothesis would provide for. Blixenkrone-Møller (19) compared the oxygen consumption of perfused livers of diabetic cats with the total acetone body production and could explain the low oxygen-acetone body ratio only by assuming that 4 molecules of acetone body were formed per molecule of fatty acid. Recently Stadie, Zapp, and Lukens (20) have found that the molecular ratio of oxygen consumed to acetone bodies produced from fatty acids by liver slices is far from that which would be expected by classical $\beta$ oxidation. These observations have led to the rise of
a theory for the production of acetone bodies by a mechanism other than $\beta$ oxidation. Hurtley (21) first proposed this hypothesis of fatty acid oxidation in which the fatty acid is attacked at alternate carbon atoms simultaneously along the whole length of the chain. He rejected the old $\beta$ oxidation hypothesis because he felt that if it were correct large amounts of the lower fatty acids intermediate between the long chain acids and the acetone bodies should be found in an intense ketosis. He overlooked the probability that these intermediate reaction compounds need only exist momentarily within the liver cells. Jowett and Quastel (16) adopted Hurtley's scheme of fatty acid oxidation which they called the "multiple alternate oxidation" hypothesis. Deuel et al. (18) propose a combination of the "multiple alternate oxidation" theory for the higher fatty acids with the usual $\beta$ oxidation theory applied to caproic and butyric acids.

There is no direct proof of any kind to support the "multiple alternate oxidation" hypothesis as the method by which acetone bodies arise during the oxidation of fatty acids. There is plenty of evidence for the successive $\beta$ oxidation of various compounds in metabolism (22). It is the most reasonable explanation of the formation of acetone bodies from caproic and butyric acids. With the evidence which has been presented in the experimental portion of this paper that acetic acid is an acetone body former the classical $\beta$ oxidation hypothesis may be modified so that it may explain all of the findings which have been cited against it.

When a fatty acid is oxidized by $\beta$ oxidation, 2 carbon atoms are dropped from the chain together. What form this cleavage takes is unknown but it is usually pictured as productive of acetic acid. If this were true and the acetic acid formed acetone bodies which we know it may do, there would be little difficulty in retaining the $\beta$ oxidation hypothesis in the light of our present knowledge. All of the carbon atoms in a fatty acid molecule with an even number of carbon atoms could yield acetone bodies (1 molecule of $C_{16} \rightarrow 4$ molecules of acetoacetic acid; 2 molecules of $C_{18} \rightarrow 9$ molecules of acetoacetic acid; etc.) which would account for the high acetone body production which made the mechanism of $\beta$ oxidation appear impossible (15, 17–19). Stadie et al. (20) support the multiple alternate oxidation hypothesis, because they find an observed molecular ratio of oxygen consumption to ketone
bodies produced in liver slices of 1.68:1 which is reasonably corrected to 1.54:1. They point out that this is not far from the theoretical ratio for palmitic acid, if submitted to multiple alternate oxidation, of 1.25:1. But it is much closer to the theoretical ratio of the modified β oxidation hypothesis which is 1.625:1. However, we must remember that rate observations of this kind, based upon mutilated bits of liver tissue in an abnormal environment, must be used with great care in drawing any quantitative conclusions. Because there was some unidentified fixed acid formed by liver slices during the oxidation of higher fatty acids, Jowett and Quastel (16) considered the possibility of acetone bodies being formed by β oxidation via acetic acid and discarded the idea because acetic acid produced acetone bodies at a slower rate than the higher fatty acids and because benzoate poisoning inhibited acetone body production by acetic (and butyric) acid but enhanced it for the higher fatty acids. Even though these observations had not been obtained from injured pieces of liver tissue which it is hard to believe may function normally, it would not appear necessary to discard the modified β oxidation hypothesis because of them.

The β oxidation hypothesis in its modified form should be retained until data are available which give better support of some other scheme. If it is correct, the acetic acid may never exist as such in appreciable amounts. For conditions under which extracellular (as far as the liver cells are concerned) acetone bodies are not required by the organism, that is, there is no ketosis, there is not even a need to suppose that the acetic acid is converted to acetone bodies. If β oxidation is the mechanism for the oxidation of fat when extracellular acetone bodies are not being formed, the acetic acid might be oxidized directly to carbon dioxide and water.

**SUMMARY**

When acetic acid is fed to a phlorhizinized dog or to fasting rats, there is an increase in the excretion of acetone bodies in the urine. Blood acetone body concentrations show that this is not due to changes in the renal threshold and the conclusion is reached that there is an increased production of acetone bodies. This is interpreted as a result of the conversion of acetic acid to aceto-acetic acid.
Acetic acid feeding during fasting results in an increase in protein catabolism.

The formation of acetone bodies by acetic acid is used to support a modified view of the \( \beta \) oxidation hypothesis of fat metabolism which meets all of the important objections which have been raised to this hypothesis.

**BIBLIOGRAPHY**

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