Little information is available on the ability of the intact, fasted rat to incorporate acetate carbon into fatty acids and cholesterol. There is evidence that a reduced food intake may lower fatty acid synthesis (1) and that tissues from fasted animals may have a similar defect (2). The intact alloxan-diabetic rat (3, 4) and liver slices from such animals (5) also exhibit decreased lipogenesis. Cholesterol formation is less altered than is fatty acid synthesis in the diabetic state (5), but information on the effect of fasting is meager.

We have investigated the effect of fasting on the ability of the intact male rat to incorporate C\textsuperscript{14} into fatty acid and cholesterol during the 1st hour following an intraperitoneal tracer dose of C\textsuperscript{14} carboxyl-labeled acetate. Respiratory C\textsubscript{14}O\textsubscript{2} collection and assay have aided in the interpretation of data on lipide C\textsuperscript{14} incorporation.

**EXPERIMENTAL**

1 week prior to use, pairs of animals were chosen from the stock colony (Sprague-Dawley strain) and hand-fed twice a day an amount of chow that would be eaten in about 1 hour. All animals showed a slight weight gain in this standardization period. Such controlled feeding made it possible to define the starvation periods within narrow limits. Four animals each were used after 1 hour and 24 hours fasting, and two each after 48, 72, 96, and 120 hours fasting.

At the end of the desired fasting period the animal was injected intraperitoneally with a tracer dose of \(1 \times 10^6\) c.p.m. per 100 gm. of weight of C\textsuperscript{14}-carboxyl-labeled acetate (6) and immediately placed in a closed metabolism system. CO\textsubscript{2}-free air was drawn past the animal and through an efficient CO\textsubscript{2} absorber containing standard NaOH. In all cases, regardless of the length of fasting, the period allowed for the metabolism of the tracer

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\+ Fellow of the Atomic Energy Commission.
acetate was 1 hour. Although one animal was used at a time, two rats chosen on a weight basis were used on the same day and the tests carried out concurrently.

After the 1 hour period the rat was chloroformed, and the entire animal, without dissection, digested in a mixture of 400 ml. of alcohol and 100 gm. of KOH per 200 gm. of tissue. Saponification under a reflux was continued for 4 hours. The digest was filtered on a coarse sintered filter, and bone fragments and insoluble residues were washed with water, alcohol, and petroleum ether. The digest and washings were concentrated to 0.5 volume on the steam bath with an air stream and cooled, and the volume was brought to 500 ml. with water. The essentially aqueous digest was extracted successively with 500, 400, 300, and 200 ml. portions of petroleum ether (b.p. 30–60°). The pooled ether extracts were washed twice with 100 ml. of N KOH, three times with 50 ml. of water, dried over Na2SO4, and evaporated by distillation to a small volume. Final traces of solvent were removed with an air stream. The non-saponifiable residue so obtained was extracted five times with small amounts of hot ethanol. Each extract was cooled and then filtered. The cold filtrate was diluted to a standard volume with alcohol, and aliquots were used for determination of cholesterol by a modified Schoenheimer-Sperry method, and for C14 radioassay.

The alkaline water phase from above and the KOH and water washes were pooled, acidified to Congo red with HCl, and extracted successively with 500, 400, 300, and 200 ml. portions of petroleum ether. Pooled ether extracts were washed three times with 100 ml. portions of water, dried over Na2SO4, and distilled. Fatty acids were separated from the residue by extracting five times with hot acetone. Each extract was cooled and filtered, and the extracts were combined. The filtrate was evaporated in a tared flask; and the residue weighed. Weighed samples of the dried fatty acids were taken for radioassay.

Although aliquots of the unsaponifiable fractions were used for combustion and radioassay, specific activities represent counts per minute per mg. of colorimetrically determined cholesterol. Labeled non-saponifiable fractions have been purified through acetate, dibromide, and digitonin derivatives, but the specific activities (counts per minute per mg. of cholesterol) were not significantly altered. Colorimetric assays compared with dried tared aliquots indicate that the non-saponifiable material contained 83 to 87 per cent cholesterol. Close adherence to the fractionation scheme above permits the recovery of 98 to 100 per cent of the cholesterol present, as tested by isotope dilution experiments with C14-labeled cholesterol.

A series of crude fatty acid fractions from the above was purified through the successive preparation of calcium and barium soaps, but little change in specific activities was found. The addition of labeled acetate to alcoholic KOH digests of control rats has failed to contaminate either fatty acid or cholesterol fractions prepared as above.
Radioassay—All lipide samples were burned, plated, and counted as infinitely thick BaC\textsubscript{14}O\textsubscript{3} samples by methods previously described (7, 8). Aliquots of the NaOH-Na\textsubscript{2}C\textsubscript{14}O\textsubscript{3} solution were precipitated and plated. C\textsubscript{12} carrier carbonate was added in amounts required for infinite thickness. All samples were counted immediately under a thin end window Geiger-Müller tube (Tracerlab, Inc., Boston, Massachusetts). In our hands such counting has an efficiency of 0.022 (counts per disintegration).

DISCUSSION

The essential data obtained from the sixteen animals fasted from 1 to 120 hours are listed in Table I.

Basic to the evaluation of C\textsubscript{14} incorporation into lipide is the necessity of knowing that fasting has not interfered with the availability of the label to the lipogenic processes. The variable, but not materially decreased, incorporation of C\textsubscript{14} into CO\textsubscript{2} indicates that fasting produced no gross defect in the conversion of the trace material to a metabolically active form. Although the label was equally available to all the animals, it is necessary in addition to consider the possible changes in dilution of the administered compound by endogenous lipide precursors. The increase found in C\textsubscript{14}O\textsubscript{2} specific activities associated with an unaltered total C\textsubscript{14} incorporation in CO\textsubscript{2} indicates less dilution of the label by endogenous CO\textsubscript{2} precursors. This decreased dilution is contrary to the concept that fasting should cause an increase in dilution of the label owing to an increase in the products of fatty acid catabolism.

No information has come to our attention as to the effects of altered metabolic states on dilution of tracer by endogenous lipide precursors. We have therefore used our CO\textsubscript{2} data as a criterion of possible changes in dilution of C\textsubscript{14} by endogenous lipide precursors. In view of the above considerations we feel that the decreased C\textsubscript{14} lipide incorporation shown in Table I indicates a similar change in lipogenesis.

After 120 hours fasting, C\textsubscript{14} incorporation into fatty acids was reduced to 30 per cent of normal. This is in contrast to the findings of Masoro et al. (2) who reported that a 24 hour fast reduced C\textsubscript{14} incorporation from glucose into fatty acids to less than one-tenth that of non-fasted controls. Decreased utilization of glucose for fatty acid synthesis may be due in part to defects in carbohydrate metabolism (9, 10), acting to depress the rate of conversion of glucose to C\textsubscript{2} fragments, and thus may not directly assess the true total lipogenic activity of the preparation. Brady and Gurin (5) found no change from normal in fatty acid C\textsubscript{14} incorporation from acetate by liver slices from normal cats fasted for 48 hours.

C\textsubscript{14} incorporation into the unsaponifiable lipide fraction decreased to about 40 per cent of normal during the fast. Liver slices from severely
### EFFECT OF FASTING UPON LIPOGENESIS

#### TABLE I

Incorporation of C\(^{14}\) from Carboxyl-Labeled Acetate into Carbon Dioxide, Fatty Acids, and Cholesterol Fractions of Fasted Rats

<table>
<thead>
<tr>
<th>Fast period, hrs.</th>
<th>1</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Body weight after fast, gm.</td>
<td>200*</td>
<td>198</td>
<td>136</td>
<td>143</td>
<td>147</td>
<td>149</td>
</tr>
<tr>
<td>Weight loss, per cent</td>
<td>0</td>
<td>22</td>
<td>28</td>
<td>26</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td><strong>CO(_2)</strong> collected, mg per hr.</td>
<td>10.7*</td>
<td>8.1-13.5</td>
<td>7.2-9.9</td>
<td>7.2-8.5</td>
<td>6.3</td>
<td>5.7</td>
</tr>
<tr>
<td>S.a.* (\times 10^{-5})</td>
<td>1.0*</td>
<td>1.36</td>
<td>1.1</td>
<td>1.73</td>
<td>1.59</td>
<td>1.32</td>
</tr>
<tr>
<td>% incorporation(\uparrow)</td>
<td>48*</td>
<td>55</td>
<td>40</td>
<td>48</td>
<td>43</td>
<td>32.5</td>
</tr>
<tr>
<td>% of 1 hr. fast</td>
<td>100</td>
<td>116</td>
<td>83</td>
<td>100</td>
<td>88</td>
<td>68</td>
</tr>
<tr>
<td><strong>Fatty acids</strong>, mg (\times 10^{-8})</td>
<td>7.2*</td>
<td>4.1-8.9</td>
<td>3.1-8.2</td>
<td>3.6-4.3</td>
<td>1.6-1.8</td>
<td>1.2-1.4</td>
</tr>
<tr>
<td>S.a.†</td>
<td>14.2*</td>
<td>10.9</td>
<td>12.4</td>
<td>22.2</td>
<td>27.5</td>
<td>17.0</td>
</tr>
<tr>
<td>% incorporation(\uparrow)</td>
<td>4.3*</td>
<td>2.3</td>
<td>2.3</td>
<td>1.7</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>% of 1 hr. fast</td>
<td>100</td>
<td>54</td>
<td>51</td>
<td>39</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td><strong>Cholesterol</strong>, mg</td>
<td>421*</td>
<td>450</td>
<td>448</td>
<td>450</td>
<td>473</td>
<td>491</td>
</tr>
<tr>
<td>S.a.§</td>
<td>481*</td>
<td>37.3</td>
<td>26.9</td>
<td>13.9</td>
<td>18.2</td>
<td>22.1</td>
</tr>
<tr>
<td>% incorporation(\uparrow)</td>
<td>0.9*</td>
<td>0.7</td>
<td>0.55</td>
<td>0.28</td>
<td>0.32</td>
<td>0.39</td>
</tr>
<tr>
<td>% of 1 hr. fast</td>
<td>100</td>
<td>80</td>
<td>60</td>
<td>31</td>
<td>34</td>
<td>41</td>
</tr>
</tbody>
</table>

* The figures represent the average (upper line) and the range (lower line).
† Specific activity = c.p.m. per mg of CO\(_2\) × Average activity administered at 1 hr. fast period
\(\) Activity administered at stated fast period
\(\) Per cent incorporation = \(\frac{\text{total activity (c.p.m.)} \times 100}{\text{total dose (c.p.m.)}}\)

§ Specific activity = c.p.m. per mg lipide × Average activity administered at 1 hr. fast period
\(\) Activity administered at stated fast period
diabetic animals demonstrate impaired cholesterol synthesis, but less severe
diabetes (5) or a restricted diet (1) affected cholesterol synthesis less
markedly. No comparable data are available on cholesterol synthesis in
the intact fasted animal.

Although the C\textsuperscript{14} incorporation into fatty acid and cholesterol fractions
showed about the same decrease during the fast, fatty acid specific activi-
ties remained essentially constant because of the proportionate decrease in
fatty acid mass. Cholesterol specific activities, on the other hand, showed
a decrease proportional to the C\textsuperscript{14} incorporation decrease, owing to the
relative constancy of the cholesterol mass. This maintenance of total body
cholesterol in the presence of decreased incorporation suggests an asso-
ciated decrease in cholesterol catabolism. It is apparent that data per-
mitting determination of total activities (and thus per cent incorporation)
as well as specific activities permit a better evaluation of fatty acid and
cholesterol synthesis and their interrelationships than do data pertaining
to specific activities alone.

Our values for changes in body, fatty acid, and cholesterol masses are
in good agreement with those of Dible (11) who fasted rats up to 95 hours.
Similar changes in body and lipide weights have been reported (12) for
humans semistarved for 24 weeks.

During our CO\textsubscript{2} collections, moderate body movements occurred in all
animals, making it unlikely that this factor alone could account for the
observed decrease in CO\textsubscript{2} production. The decrease to one-half observed
in the amount of CO\textsubscript{2} produced per hour may be due to both decreased
metabolic rate and lowering of the respiratory quotient. It is interesting
that humans semistarved for 24 weeks exhibited a 60 per cent decrease in
O\textsubscript{2} consumption (12).

SUMMARY

C\textsuperscript{14}-carboxyl-labeled acetate was given intraperitoneally to fasted adult
male rats, and the animals were allowed to metabolize the dose for 1 hour.
Those fasted 120 hours lost about 25 per cent body weight and about 75
per cent of their fatty acids. The cholesterol content, however, remained
essentially constant. The amount of C\textsuperscript{14} appearing in respired CO\textsubscript{2} did
not change materially as the fast progressed from 1 to 120 hours. C\textsuperscript{14}
appearing in fatty acids and cholesterol decreased to about one-third in
this period.

BIBLIOGRAPHY

185, 845 (1950).
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