FATTY ACID METABOLISM AND HEPATIC LIPOGENESIS*

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The conversion of octanoate-1-C\textsuperscript{14} to radioacetoacetate and the effect of pyruvate on this process are dependent on the K\textsuperscript+ concentration in the incubation medium (1). These results prompted this investigation in which the incorporation of octanoate-1-C\textsuperscript{14} into fatty acids and cholesterol was determined in the presence of non-radioactive additions of pyruvate, succinate, and fumarate. Malonate was used as an aid in evaluating the relationship between the simultaneous metabolism of octanoate and the de novo synthesis of fatty acids. The lipogenic effects of high and low K\textsuperscript+ concentrations in the medium were also studied.

EXPERIMENTAL

Treatment of Animals—Male rats,\textsuperscript{1} with a mean body weight of 200 ± 22 gm., were used in these studies and were fed a stock diet\textsuperscript{2} ad libitum for varying periods of time. The rats were sacrificed by a sharp blow on the head and their livers were quickly excised. Liver slices were prepared free-hand with a thin razor blade.

Incubation Procedure—The incubation procedure has been described in an earlier publication from this laboratory (2). Octanoate-1-C\textsuperscript{14} (1.51 mm per liter, final concentration) was added to each flask. The final concentrations of the other additions were as follows: Na pyruvate, Na succinate, and Na fumarate, 7.75 mm per liter, and for Na malonate 5.84 mm per liter. The composition of the media employed will be found in Tables I and II. The high K\textsuperscript+ medium contained 70 m.eq. per liter, final concentration, while the corresponding value for the low K\textsuperscript+ medium was 7 m.eq. per liter.

Analytical Procedure; Respired C\textsuperscript{14}O\textsubscript{2}—Following incubation, the vial containing KOH for carbon dioxide absorption was removed and immediately stoppered. Aliquots of the alkaline solution were mounted directly

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† Holder of a Fellowship in the Medical Sciences administered by the National Research Council for the Rockefeller Foundation.

1 Charles River Breeding Laboratories, Inc., Brookline, Massachusetts.
2 Purina chow checkers, Ralston Purina Company, St. Louis, Missouri.
in stainless steel cups and counted immediately thereafter with an end
window Geiger-Müller tube. Experiments have shown\(^3\) that C\(^{14}\)O\(_2\) deter-
minations by this technique gave data comparable to those obtained with
BaCO\(_3\) mounts, provided counting was carried on immediately after prep-
paration of the direct mounts. The changes in the RCO\(_2\) pattern\(^4\) follow-
ing supplementation in vitro were also comparable by the two methods.
Counting data for this and all other fractions were expressed as total counts
per minute per gm. of tissue (wet weight) per hour.

**Acetoacetate-C\(^{14}\)OOH**—The medium, including washings from the slices,
was acidified and treated with aniline citrate to decarboxylate the aceto-
acetate contained therein\(^2\). The released carbon dioxide was trapped
in KOH, and C\(^{14}\)O\(_2\) determinations were made as indicated above.

**Cholesterol**—The slices were saponified in alcoholic KOH, and the saponi-
fication mixture was then extracted three times with petroleum ether.
These extracts were combined and washed once with 1 N KOH, twice with
water, and once with a solution of Na palmitate (10 mg. per ml.). The
latter was done to minimize any loss of the long chain fatty acids. The
washings were added to the extracted saponification mixture, which was
then reduced to a volume of about 1 to 2 ml.

Aliquots of the petroleum ether extracts were mounted directly and
counted. The efficiency of the cholesterol extraction was determined in
separate aliquots by forming the digitonides. The counting data obtained
with these derivatives did not significantly differ from those obtained with
the petroleum ether extracts.

**Fatty Acids**—The saponification mixture was deproteinized by the copper-
lime method described by Lehninger and Smith\(^3\). The precipitate
was treated according to the method of Brady and Gurin\(^4\), with one
modification. Acidification and reprecipitation with alkali were repeated
twice. Pilot experiments indicated that repetition of these proced-
ures was necessary to eliminate effectively contamination of the long chain
fatty acid fraction by short chain acids entrapped within the precipitate.
The final precipitate was acidified with 6 N HCl and extracted with petro-
leum ether. Aliquots of the extract were mounted directly as described
by Entenman \emph{et al.}\(^5\) and counted. This fraction, LFA, contained fatty
acids with 10 or more carbon atoms per molecule.

The supernatant fluid and washings obtained from centrifugation of the
precipitate were combined, acidified with 6 N HCl, and extracted with petro-
leum ether. Aliquots of the extract were mounted directly as indi-
cated above and counted. This fraction, SFA, contained fatty acids hav-

\(^3\) Lyon, I., unpublished experiments.

\(^4\) The following symbols are used: RCO\(_2\), respiratory C\(^{14}\)O\(_2\); ACO\(_2\), acetoacetate-
C\(^{14}\)OOH; LFA, long chain fatty acid-C\(^{14}\); SFA, short chain fatty acid-C\(^{14}\); CHOL,
cholesterol-C\(^{14}\).
ing fewer than 10 carbon atoms per molecule. The radioactivity in this fraction includes that in the de novo short chain fatty acids and unutilized octanoate. Preliminary work indicates that the latter comprises approximately 50 per cent of the SFA fraction.

RESULTS AND DISCUSSION

The C₂ units from octanoate are available for various condensation reactions leading to the formation of respiratory CO₂ (RCO₂), acetoacetate (ACO₂), long and short chain fatty acids (LFA and SFA), and cholesterol (CHOL). The data reported in this paper indicate that the addition of pyruvate, succinate, or fumarate results in a diversion of C₂ units from ACO₂ to LFA. The simultaneous increase in RCO₂ counts may be a reflection of this process. A reduction in the recovery of labeled C₂ units in SFA occurs with pyruvate, but an increase in SFA is observed with succinate (not significant) or fumarate. This difference might be due to the formation of short chain fatty acid derivatives or other substances which estimate as SFA. Succinate or fumarate supplementation did not affect CHOL recoveries, although pyruvate addition lowered CHOL counts.

In Series I, Tables I and II, following pyruvate supplementation the decreased availability of C₂ units for condensation reactions (other than that forming active citrate) was suggested by the lower C¹⁴ recoveries in ACO₂, SFA, and CHOL. Thus the C₂ distribution pattern favored the increase observed in LFA. The addition of succinate (Series II, Tables I and II) resulted in an inverted C₂ distribution between ACO₂ and LFA (compared to that of the control), C¹⁴ recoveries in ACO₂ being lowered while those in the LFA fraction were elevated. The other fractions showed no significant change. Fumarate addition (Series III, Tables I and II) to the incubation medium caused a decrease in ACO₂ and an increase in SFA. The other fractions were unaffected, although C¹⁴ recoveries in LFA tended toward higher values. The increase in short chain fatty acid counts was not great enough to account for the decrease in C¹⁴ activity in ACO₂.

The fumarate effect on the higher fatty acids was not as marked as that as previously shown, the acetone moiety of acetoacetate produced under these conditions has approximately 60 to 70 per cent of the activity of the carboxyl moiety. Malonate inhibition results in parallel increases in both moieties of the acetoacetate molecule (6). Furthermore, supplements such as pyruvate, succinate, or fumarate (unpublished observations in this laboratory) effect a parallel decrease in both fractions of acetoacetate. Although no determinations of the acetone moiety were made in the present studies, it should be borne in mind that any change observed in activity of the carboxyl group would be approximately doubled for the total activity in acetoacetate.

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With this single exception, all differences between groups involving either an increase or a decrease are significant at a probability level of 5 per cent with a t test (7).
<table>
<thead>
<tr>
<th>Series No.</th>
<th>Group No.</th>
<th>Supplement</th>
<th>Ratio of supplemented flask to control flask for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RCO₂</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>Pyruvate</td>
<td>1.17 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Malonate</td>
<td>0.36 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Pyruvate + malonate</td>
<td>0.45 ± 0.07</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>Succinate</td>
<td>1.05 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Malonate</td>
<td>0.30 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Succinate + malonate</td>
<td>0.39 ± 0.06</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>Fumarate</td>
<td>1.10 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Malonate</td>
<td>0.38 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Fumarate + malonate</td>
<td>0.44 ± 0.03</td>
</tr>
</tbody>
</table>

Each flask contained 750 mg (wet weight) of liver slices, octanoate-1-Cl₄, pyruvate, succinate, fumarate, or malonate added singly or in combination with the latter substance, Krebs-phosphate buffer, pH 7.4, made up with isotonic NaCl and KCl (1:1 volume per volume). The low K⁺ medium used in Series IV had the following composition: 0.9 per cent NaCl (100 parts), 1.15 per cent KCl (4 parts), 2.11 per cent K₂HPO₄ (1 part), 1.85 per cent MgSO₄ (1 part), PO₄ buffer (12 parts), pH 7.4 (8). That referred to above, including “High K⁺ control” and “High K⁺ + succinate” of Series IV, was high in K⁺. The ratios in this table represent comparisons between the total counts in a given fraction and the total activity in the corresponding fraction of the control group. Thus the effects of the supplements on each fraction may be noted. Total volume, 10 ml.; incubation time, 1 hour; temperature, 37.5°C; gas phase, 100 per cent O₂.

* Series I, II, III, and IV included six, five, four, and three rats, respectively.
† Ratio ± standard deviation of the mean. In a given series the control value in a particular column was assigned a value of 1.00. The other values of that series appearing in the same column were made relative to the control value.
‡ The final concentration of pyruvate, succinate, or fumarate (Na salts) in each flask was 7.75 mM per liter.
§ The final concentration of Na malonate in each flask was 5.84 mM per liter.
of pyruvate or succinate. Similar differences in effect were noted by Bloch (9). Using \( \text{CH}_3\text{C}^4\text{OONa} \) and \( \text{D}_2\text{O} \), he found that fatty acid synthesis was stimulated strongly by pyruvate or oxalacetate supplementation and less strongly by the addition of fumarate. In a series of studies with rat liver suspensions in a phosphate buffer, Lehninger (10) demonstrated a quantitative conversion of octanoate into acetoacetate in the absence of oxalacetate or oxalacetate precursors. In the presence of

### Table II

<table>
<thead>
<tr>
<th>Series No.</th>
<th>Group No.</th>
<th>RCO₂</th>
<th>ACO₂</th>
<th>LFA</th>
<th>SFA</th>
<th>CHOL</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>1.0</td>
<td>6.1</td>
<td>0.9</td>
<td>1.1</td>
<td>0.8</td>
<td>9.9</td>
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<td>1</td>
<td>1.2</td>
<td>4.4</td>
<td>1.2</td>
<td>0.6</td>
<td>0.6</td>
<td>8.0</td>
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<td>2</td>
<td>0.4</td>
<td>9.5</td>
<td>0.8</td>
<td>1.8</td>
<td>0.4</td>
<td>12.9</td>
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<tr>
<td></td>
<td>3</td>
<td>0.5</td>
<td>8.2</td>
<td>1.6</td>
<td>1.4</td>
<td>0.5</td>
<td>12.2</td>
</tr>
<tr>
<td>II</td>
<td>Control</td>
<td>1.0</td>
<td>3.4</td>
<td>0.4</td>
<td>1.1</td>
<td>0.7</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.1</td>
<td>2.1</td>
<td>0.6</td>
<td>1.4</td>
<td>0.7</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.4</td>
<td>5.8</td>
<td>0.4</td>
<td>1.7</td>
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<td>8.7</td>
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<td>3</td>
<td>0.4</td>
<td>5.7</td>
<td>0.8</td>
<td>1.3</td>
<td>0.4</td>
<td>8.6</td>
</tr>
<tr>
<td>III</td>
<td>Control</td>
<td>1.0</td>
<td>5.2</td>
<td>0.3</td>
<td>0.7</td>
<td>0.6</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.1</td>
<td>2.5</td>
<td>0.3</td>
<td>0.9</td>
<td>0.6</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.4</td>
<td>8.1</td>
<td>0.3</td>
<td>1.2</td>
<td>0.3</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.4</td>
<td>6.7</td>
<td>0.5</td>
<td>1.1</td>
<td>0.4</td>
<td>9.1</td>
</tr>
<tr>
<td>IV</td>
<td>High K⁺ control</td>
<td>1.0</td>
<td>3.7</td>
<td>0.3</td>
<td>1.0</td>
<td>0.4</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Low &quot; &quot;</td>
<td>1.3</td>
<td>3.7</td>
<td>0.2</td>
<td>2.3</td>
<td>0.4</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>&quot; K⁺ + succinate&quot;</td>
<td>1.5</td>
<td>2.2</td>
<td>0.3</td>
<td>0.8</td>
<td>0.5</td>
<td>5.3</td>
</tr>
</tbody>
</table>

All the figures in a given series are relative to the control RCO₂ of the series which has been assigned a value of 1.0. In Series IV, all the figures are relative to RCO₂ of the "High K⁺ control." RCO₂ was chosen as the reference fraction primarily since it was the most consistent in its pattern response to the various supplements. The ratios in this table represent comparisons between the total counts in a given fraction and the total activity in the control RCO₂ of the series. In this way, the distribution pattern of the C₂ units may be seen.

fumarate, Krebs cycle intermediates accumulated with a parallel decrease in the formation of acetoacetate.

The presence of malonate in the medium caused a depression in RCO₂ (Group 2, Series I, II, III, Tables I and II). There was an accompanying but unequal rise in ACO₂ and SFA. The increase in SFA, while LFA remained unchanged, suggested that additional substances estimating as SFA may have formed. The recoveries of C₁⁴ in CHOL indicated some link between RCO₂ and cholesterogenesis. Over-all utilization of C₂ units by the slice was high in the presence of the inhibitor (Table II). When malonate with pyruvate, succinate, or fumarate was added to the medium
(Group 3, Series I, II, and III, Tables I and II), RCO₂ values remained unchanged (versus malonate alone), indicating the effectiveness of the malonate block. However, there was a simultaneous diversion of C₂ units from both ACO₂ and SFA and a marked incorporation of these units into LFA. The further addition of pyruvate, succinate, or fumarate did not alter the C¹⁴ recoveries in CHOL observed with the inhibitor alone.

In the presence or absence of malonate, the addition of pyruvate, succinate, or fumarate resulted in an increased conversion of radiooctanoate into LFA-C¹⁴. A concomitant but non-equivalent decrease in acetoacetate-C⁴⁰₀H was observed. The marked depression in respiratory activity due to malonate was not reflected in the recovery of C¹⁴ in the LFA fraction. Since the incorporation of the labeled C₃ acid into SFA varied considerably under the experimental conditions, it was difficult to discern any relationship between this fraction and LFA or ACO₂. Hence, the synthesis of LFA from octanoate seemed to be inversely related to the formation of acetoacetate primarily. It is interesting to note that, in contrast to LFA, cholesterogenesis from octanoate paralleled the formation of ACO₂ and was inversely related to ACO₂ formation (Table I, Series I, II, and III). The latter observation may indicate C₄ utilization in cholesterol synthesis (11).

Brady and Gurin (4) found radioactivity in long chain fatty acids of liver when slices were incubated with labeled octanoate (5800 to 14,500 counts per flask) in a Krebs-Ringer-bicarbonate medium, but not in a Krebs or Lehninger phosphate medium. They concluded that the Krebs-Ringer-bicarbonate solution and the intact liver cell are necessary for long chain fatty acid synthesis from octanoate. Following the incubation of liver slices in Krebs-phosphate buffer containing deuterioacetate or D₂O, Bloch, Borek, and Rittenberg (12) isolated fatty acids having small but significant isotope concentrations. According to the method of calculation used by Brady and Gurin (4), about 2½ times more octanoate was incorporated into fatty acids in the high K⁺ medium used in the experiments described herein than in the bicarbonate medium employed by those investigators.

Although strain differences may have contributed to these contradictory results, a study was made of the effect of the high K⁺ medium versus Krebs-Ringer-phosphate buffer, low in K⁺ concentration, on the incorporation of octanoate into fatty acids. Each flask contained 160,000 counts at the start of the incubation. In the low K⁺ medium, incorporation was reduced to one-half that observed in the high K⁺ medium (Series IV, Tables I and II). An average of 1350 (range, 1230 to 1670) counts was found in LFA in the low K⁺ medium; the corresponding value for the high K⁺ medium was 2490 (range, 2400 to 2570) counts. It appears that octanoate
incorporation into long chain fatty acids occurs in a phosphate medium, but in such a medium it is sensitive to the K+ concentration.

SUMMARY

1. The incorporation of octanoate-1-C14 into respiratory CO2 (RCO2), acetoacetate (ACO2), long and short chain fatty acids (LFA and SFA), and cholesterol (CHOL) was studied in rat liver slices.

2. Changes in the distribution pattern of labeled C2 units derived from octanoate were noted following supplementation of the incubation medium with pyruvate, succinate, or fumarate alone or in combination with malonate.

3. These changes indicated that long chain fatty acid synthesis was primarily related in an inverse manner to the amount of acetoacetate formed. Short chain fatty acids influenced or were influenced by this relationship secondarily.

4. LFA recoveries were not affected by malonate alone, although RCO2 was markedly depressed; the C14 content of ACO2 and SFA increased. There appeared to be some link between cholesterol synthesis and respiratory activity.

5. The addition of malonate with pyruvate, succinate, or fumarate resulted in a diversion of C2 units from ACO2 and SFA into LFA. RCO2 and CHOL recoveries were unchanged from those noted in the presence of malonate alone.

6. The conversion of octanoate into long chain fatty acids occurs in a phosphate medium, but in such a medium lipogenesis is sensitive to the K+ concentration.

7. Changes observed in the distribution pattern of C2 units among the fractions determined are discussed.

BIBLIOGRAPHY


7 Toward the completion of this paper, data published by Curran and Clute (13) demonstrated a higher incorporation of acetate-C14 into fatty acid-C14 by liver cell clusters when a PO4 medium contained higher levels of K+. This result was independent of pH. In contrast, however, Mg2+ stimulated fatty acid synthesis at pH 6.5 but not at pH 7.4. Hence, the Mg2+ influence may reasonably be attributed to a shift in pH. Studies concerning the effects of Mg2+ and other ions on lipogenesis are being continued in this laboratory.
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