Metabolism of Pyruvate and L-Lactate by Rat Adipose Tissue*

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SUMMARY

The metabolism of L-lactate and pyruvate, labeled uniformly with 14C, by epididymal fat pad tissue of fasted and fasted-refed rats was studied. In tissue of fasted-refed rats, pyruvate and L-lactate were utilized at equal rates. L-Lactate was nearly all converted into CO2 and fatty acids, and pyruvate into CO2, fatty acids, and L-lactate. The incorporation of 14C into glycerol was very low. L-Lactate was a better precursor of fatty acids than pyruvate. In tissue of fasted rats, pyruvate utilization was depressed, but still proceeded at an appreciable rate, and pyruvate carbon was incorporated into fatty acids and glycerol. The utilization of L-lactate by tissue of fasted rats was very low, and there was insignificant incorporation into lipids.

Addition of unlabeled pyruvate or glucose to tissue of fasted rats metabolizing uniformly labeled L-lactate increased CO2 incorporation into CO2 and very markedly into lipid, but there was no net uptake of L-lactate. The 14C yields reflect exchange between labeled L-lactate and unlabeled pyruvate. Addition of malate, aspartate, α-ketoglutarate, and glutamate did not increase the utilization of uniformly labeled L-lactate-14C by tissue of fasted rats, but aspartate and α-ketoglutarate when added together stimulated L-lactate uptake, and incorporation of 14C into CO2 and lipids. Addition of oralactate and propionate increased to some extent 14C incorporation into CO2 and lipid, without appreciably stimulating L-lactate uptake. Addition of α-ketoisovalerate or α-acetooacetate stimulated incorporation of L-lactate carbon into CO2 and lipids and net utilization of L-lactate by tissue of fasted rats. There was accumulation of α-hydroxyisovalerate but not of β-hydroxyisovalerate. Addition of acetate or β-hydroxyisovaleric acid was without effect.

We conclude that in adipose tissue of rats there is no transfer of hydrogen equivalents from cytoplasm to mitochondria. When lipogenesis is pronounced, cytoplasmic hydrogen equivalents are all used in reductive biosynthesis; in the fasted state DPNH accumulates in the absence of lipogenesis, hydrogen equivalents from L-lactate oxidation cannot be utilized, and no L-lactate oxidation to pyruvate can occur.

The metabolism of 14C-labeled pyruvate by rat epididymal fat pad tissue was first studied by Rose and Shapiro (1). They found that pyruvate was oxidized to CO2 and incorporated into lipid. The incorporation into lipid was depressed in tissue of fasted rats. These studies were extended by Winegrad and Renold (2) and Christophe et al. (3), who found pyruvate incorporated both in fatty acids and lipid glycerol, by Ballard, Hanson, and Levilie (4), and more recently by Kneer and Ball (5).

The concentration of L-lactate in blood and tissues is much higher than that of pyruvate, but the metabolism of L-lactate by adipose tissue has not been thoroughly studied. L-Lactate is more reduced than pyruvate and hence should in theory serve as a more efficient precursor for fatty acids than pyruvate. The present study compares metabolism of pyruvate and L-lactate in rat epididymal fat tissue and under varying dietary regimes. We find that under conditions of maximal lipogenesis, as in tissue of fasted-refed rats, L-lactate and pyruvate are utilized at equal rates, but L-lactate is a more efficient precursor of fatty acids than pyruvate. In tissue of fasted rats, on the other hand, L-lactate is metabolized very poorly, whereas pyruvate metabolism is still appreciable. This difference in the metabolism of pyruvate and L-lactate is interpreted in terms of the mechanism of hydrogen transfer in adipose tissue. We conclude that hydrogen equivalents formed in the cytoplasm in rat adipose tissue cannot be transferred into mitochondria.

METHODS

Animals—Male rats of the Wistar strain, 120 to 200 g in weight, were fed either a regular pellet diet, containing about 12% fat, or a high carbohydrate fat-free pellet diet (Nutritional Biochemicals). Fasted-refed rats were starved for 2 or 3 days and refed the fat-free diet for 2 days. Fasted rats were kept on regular diet and food was withdrawn 2 days before they were killed.

Assay of Substrates—L-Lactate and pyruvate were assayed enzymatically (6, 7). α-Ketoisovalerate and α-hydroxyisovalerate were assayed under conditions used for pyruvate and L-lactate, respectively, with L-lactate dehydrogenase from pig heart.
**Effect of diet on utilization of \( \alpha \)-lactate and pyruvate by rat adipose tissue**

Epididymal fat pad segments (150 to 250 mg) from one tissue pool were incubated in parallel with \( \alpha \)-lactate and pyruvate uniformly labeled with \( ^{14} \)C in 2 ml of Krebs-Henseleit bicarbonate buffer (95% O\(_2\)-5% CO\(_2\)) for 3 hours at 37°C. The enzymes were purchased either from Sigma or from Boehringer-Mannheim Corporation and then the enzyme from pig heart, which reacts with the \( \beta \)-hydroxy- or ketobutyrate, was added. A small correction for the slow oxidation of these acids by the enzyme from muscle was applied (7). \( \beta \)-Hydroxybutyrate was determined according to Williamson and Mellanby (8). The enzymes were purchased from Sigma or from Boehringer-Mannheim Corporation (San Francisco, California).

**Labeled Substrates**—Pyruvate-\( ^{14} \)C was purchased in 50 \( \mu \)Ci evacuated ampoules from New England Nuclear. After opening, any unused material was lyophilized and stored under vacuum in the deep freeze. Even under these conditions deterioration of the samples occurs. Samples more than 2 weeks old were repurified by paper chromatography in butanol-acetic acid-water (100:25:50). Pyruvate seems to form decomposition products during chromatography, and normal criteria of radioactivity as used with other labeled compounds are difficult to apply with pyruvate-\( ^{14} \)C.

Pyruvate plus \( \alpha \)-ketobutyrate or \( \alpha \)-lactate plus \( \alpha \)-hydroxybutyrate were determined simultaneously in one cuvette. Pyruvate and \( \alpha \)-lactate (or \( \alpha \)-lactate) were first determined with the enzyme from muscle, and then the enzyme from pig heart, which reacts with the hydroxy- or ketobutyrate, was added. A small correction for the slow oxidation of these acids by the enzyme from muscle was applied (7). \( \beta \)-Hydroxybutyrate was determined according to Williamson and Mellanby (8). The enzymes were purchased either from Sigma or from Boehringer-Mannheim Corporation (San Francisco, California).

**Results**

**TABLE I**

<table>
<thead>
<tr>
<th>Experiment and conditions</th>
<th>Concentration of lactate or pyruvate when added</th>
<th>Utilization*</th>
<th>Incorporation into lipids from</th>
<th>Pyruvate</th>
<th>( \alpha )-Lactate</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Fatty acids</td>
<td>Glycerol</td>
<td>Fatty acids</td>
<td>Glycerol</td>
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<tr>
<td></td>
<td></td>
<td>( \mu )moles/( \mu )l/3 hr</td>
<td>( \mu )atoms/( \mu )l/3 hr</td>
<td>( \mu )atoms/( \mu )l/3 hr</td>
<td>( \mu )atoms/( \mu )l/3 hr</td>
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<td>Fat-free diet; fasted refed</td>
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<td></td>
<td></td>
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<tr>
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<td></td>
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<td>1B</td>
<td>20</td>
<td>48</td>
<td>46</td>
<td>36</td>
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<td>Regular diet ad libitum</td>
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<tr>
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<td>10</td>
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</tbody>
</table>

* Determined by enzymatic assay.

† Not determined.

\( \alpha \)-Lactate-\( ^{14} \)C was either purchased or prepared from pyruvate-\( ^{14} \)C by reduction with DPNH and lactate dehydrogenase. The \( \alpha \)-lactate was purified by paper chromatography. Frozen solutions of \( \alpha \)-lactate are stable for many weeks.

**Incubation, Fractionation, and Analysis**—Incubation of adipose tissue was performed in Krebs-Henseleit bicarbonate buffer, in an atmosphere of 95% O\(_2\)-5% CO\(_2\), for 3 hours at 37°C. Methods of tissue preparation, incubation, isolation of \( ^{14} \)CO\(_2\), fatty acids, and lipid glycerol, and radioassay have been previously described (9, 10). Uptake of \( \alpha \)-lactate and pyruvate was determined by enzymatic assay. Aliquots of the aqueous extract were applied to Dowex 1-acetate columns and eluted with either 1 N acetic acid to obtain \( \alpha \)-lactate, or a linear gradient of water to 6 N formic acid to obtain \( \alpha \)-lactate, pyruvate, and other compounds.

**RESULTS**

**Metabolism of Pyruvate and \( \alpha \)-Lactate by Adipose Tissue of Fasted and Fed Rats**—In our studies we have mainly used tissue from fasted-reared rats receiving a fat-free diet, and tissue from fasted rats. These conditions represent metabolic extremes with respect to fat synthesis. Adipose tissue of rats fed a high carbohydrate fat-free diet, and especially of fasted-reared rats, is characterized by the capacity for maximal lipogenesis. In tissue of fasted rats, fatty acid synthesis is limited and lipolysis predominates (9, 10). In between these extremes a range of intermediate states may be obtained according to the dietary regimen. It is convenient to designate the two extreme metabolic conditions as the "lipogenic" and "lipolytic" states, respectively.

In Table I the effect of dietary status on the utilization of pyruvate and \( \alpha \)-lactate is shown. Under conditions of high lipogenesis, the utilization of these acids was about equal. Utilization of either pyruvate or \( \alpha \)-lactate was 60 to 90 \( \mu \)moles per g per 3 hours in tissue of fasted-reared rats and somewhat less in those fed a fat-free diet ad libitum. (These rates are not maximal, as utilization increases with higher substrate concentration.) In tissue of fasted rats, pyruvate utilization was reduced, but still proceeded to a considerable extent. However, utilization of \( \alpha \)-lactate fell to very low levels, 0.1 that of pyruvate.

In Table II the distribution of \( ^{14} \)C in products from pyruvate-\( ^{14} \)C and \( \alpha \)-lactate-\( ^{14} \)C is reported. The results are expressed as specific yields, that is, percentage of \( ^{14} \)C recovered in products. The major products from the metabolism of pyruvate-\( ^{14} \)C were \( ^{14} \)CO\(_2\), lipid, and \( \alpha \)-lactate. Also 2 to 3% were found in soluble products, mainly amino acids, 0.5 to 1% in protein, and negligible amounts in glycogen. A small but variable fraction was eluted from the column after pyruvate, as observed also by Van Korff (11). This probably represents a decomposition product, and the \( ^{14} \)C in this peak was excluded in the calculation of the specific yields.

When \( \alpha \)-lactate was the substrate, the major products were \( ^{14} \)CO\(_2\) and lipid and only 2 to 5% in pyruvate. In most experiments the \( ^{14} \)C in the minor fractions was neglected in the calculation of specific yields, and the utilized \( ^{14} \)C was taken as that in \( ^{14} \)CO\(_2\) and lipid, and \( \alpha \)-lactate from pyruvate.

Under conditions of high lipogenesis, \( \alpha \)-lactate-\( ^{14} \)C was a considerably better precursor for fatty acids than pyruvate. Incorporation of \( ^{14} \)C from \( \alpha \)-lactate into fatty acids slightly exceeded or was about equal to that incorporated into \( ^{14} \)CO\(_2\). From

\( ^{14} \)CO\(_2\), lipid, and \( \alpha \)-lactate. Also 2 to 3% were found in soluble products, mainly amino acids, 0.5 to 1% in protein, and negligible amounts in glycogen. A small but variable fraction was eluted from the column after pyruvate, as observed also by Van Korff (11). This probably represents a decomposition product, and the \( ^{14} \)C in this peak was excluded in the calculation of the specific yields.

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pyruvate-\(^{14}C\), \(^{14}CO_2\) yields were 1.5 to 2 times that in fatty acids. Very little \(^{14}C\) from both precursors was found in lipid glycerol. Epididymal fat pad tissue from either fasted-refed rats or from those fed a fat-free diet was found to contain a considerable amount of glycogen which was utilized during incubation. Experiments to be reported elsewhere indicate that under these conditions practically all of the lipid glycerol is derived from glycogen.

In tissue of fasted rats metabolism of pyruvate-\(^{14}C\) was completely different. Formation of \(L\)-lactate was greatly increased and synthesis of lipids was depressed. Two-thirds of the \(^{14}C\) in lipids was derived from fatty acids. Incorporation of pyruvate carbon into fatty acids in fasted rats was of the order of 5% of that in fasted-refed animals. On the other hand, incorporation into glycerol in fasted rats was 50 to 10 times higher than in fasted-refed animals. Fasting nearly completely abolished lipid synthesis from \(L\)-lactate, and 95% of the utilized \(^{14}C\) was in \(CO_2\). Incorporation of carbon from \(L\)-lactate into fat was 1 to 2% that from pyruvate. Rats fed a regular diet containing 12yo fat showed an intermediate pattern between the extremes. Results with this group were quite variable.

When epinephrine was added to adipose tissue from fed rats, the tissue is in the lipolytic state and the metabolic pattern from pyruvate-\(^{14}C\) and \(L\)-lactate-\(^{14}C\) resembled that with fasted animals (Table III). Utilization of pyruvate was not affected, but that of \(L\)-lactate was depressed to less than half of the values without epinephrine. Incorporation into lipids was depressed with both substrates, but more extensively with \(L\)-lactate.

The intractability of tissue in the lipolytic state to utilize \(L\)-lactate is not due to any inhibition of lactate dehydrogenase. This is shown in Table IV, which shows the effect of added pyruvate. Addition of pyruvate in equimolar amounts increased \(^{14}CO_2\) production from \(L\)-lactate-\(^{14}C\) 3-fold and lipogenesis 20 to 70-fold. However, as determined analytically, there was an increase in the amount of \(CO_2\) produced. The data of Table IV indicate that the increased \(^{14}CO_2\) yield from \(L\)-lactate-\(^{14}C\) in the presence of pyruvate does not represent a stimulation of \(L\)-lactate utilization, but is due to an exchange of \(L\)-lactate carbon with that of pyruvate, catalyzed by lactate dehydrogenase.

A number of compounds were tested for their effects in stimulating \(L\)-lactate metabolism in tissue of fasted rats. The addition of glucose increased \(^{14}CO_2\) formation and rather markedly the \(^{14}C\) yield in lipids. However, there was no net uptake of \(L\)-lactate and frequently a lactate formation. The effect of glucose on \(^{14}C\) yields represents essentially an exchange reaction between pyruvate derived from glucose and that from \(L\)-lactate. Addition of oxaloacetate stimulated \(^{14}CO_2\) yields from \(L\)-lactate, and to a limited extent \(^{14}C\) incorporation into lipids, but without net uptake of \(L\)-lactate. It is likely that this effect of exogenous oxaloacetate is due to the increase in the amount of pyruvate, formed from extracellular oxaloacetate breakdown.

The addition of succinate, malate, \(\alpha\)-ketoglutarate, aspartate, and glutamate was without significant effect on \(L\)-lactate-\(^{14}C\) metabolism. However, when aspartate and \(\alpha\)-ketoglutarate were added together, the incorporation into \(^{14}CO_2\) and lipids was markedly stimulated (Table V), and there was net \(L\)-lactate utilization. It is likely that under these conditions aspartate provides, by transamination, cytoplasmic oxaloacetate. The oxaloacetate would be reduced to malate or, via the pathway sequence outlined by Chakraberty and Loveloe (12), be converted to glycerol. The synthesis of a molecule of \(\alpha\)-glycerophosphate requires 2 molecules of cytoplasmic DPNH. No accumulation of malate could be detected by enzymatic assay. It is likely that malate is deaminated to pyruvate and TPNH, which is used in the synthesis of fatty acids. Generation of DPN by the above reactions is accompanied by the conversion of \(L\)-lactate to pyruvate, which in turn is metabolized in mitochondria.

The finding that no significant stimulation of \(L\)-lactate metab-
TABLE IV

Effect of pyruvate on L-lactate utilization by adipose tissue of fasted rats

In Experiment I, 250 mg, and in Experiment II, 296 mg, of epididymal fat pad tissue (pooled from six rats) were incubated in 1 ml of Krebs-Henseleit bicarbonate buffer (gas phase, 95% O₂-5% CO₂) for 3 hours at 37°C.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>L-Lactate-U-14C</th>
<th>Pyruvate-U-14C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>L-Lactate-U-14C</td>
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<td>0.5</td>
</tr>
<tr>
<td>Pyruvate-U-14C</td>
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<td>n.d.*</td>
</tr>
<tr>
<td>Pyruvate-U-14C</td>
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<td>n.d.*</td>
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<td>n.d.*</td>
</tr>
<tr>
<td>Pyruvate-U-14C</td>
<td>0</td>
<td>n.d.*</td>
</tr>
</tbody>
</table>

TABLE V

Effect of addition of substrates on L-lactate metabolism by adipose tissue of fasted rats

Adipose tissue (100 or 200 mg) was incubated with 1 to 2 µCi of L-lactate-U-14C in 1 ml of Krebs-Henseleit bicarbonate buffer (95% O₂-5% CO₂). The lactate concentration was 5 or 10 mM and the concentration of the added compounds was equal to that of lactate. The lactate utilization was determined by enzymatic assay.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Conversion of L-lactate-U-14C to CO₂</th>
<th>L-Lactate utilization</th>
<th>Pyruvate utilization</th>
<th>CO₂</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td>-0.8 ± 0.7 I 5.5 ± 1 I 0.26 ± 0.19</td>
<td>-0.8 ± 0.7</td>
<td>-0.8 ± 0.7</td>
<td>7.0</td>
<td>0.2</td>
</tr>
<tr>
<td>+a-ketobutyrate</td>
<td>+1.3 ± 1.9 I 7.9 ± 3.2</td>
<td>+1.3 ± 1.9</td>
<td>+1.3 ± 1.9</td>
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<td>14</td>
</tr>
<tr>
<td>+a-ketobutyrate</td>
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<td>+1.5 ± 1.8</td>
<td>+1.5 ± 1.8</td>
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<td>11</td>
</tr>
<tr>
<td>+a-ketobutyrate</td>
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<td>+1.7 ± 1.9</td>
<td>25</td>
<td>14</td>
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</tbody>
</table>

TABLE VI

Effect of α-ketobutyrate on L-lactate metabolism in adipose tissue

Slices of adipose tissue (250 mg) were incubated in 1 ml of Krebs-Henseleit bicarbonate buffer (95% O₂-5% CO₂) with about 2 µCi of L-lactate-U-14C. Concentration of L-lactate and α-ketobutyrate was 5 mM with tissue of fasted rats and 20 mM with tissue of refed rats.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Conversion of L-lactate-U-14C to CO₂</th>
<th>L-Lactate utilization</th>
<th>Pyruvate utilization</th>
<th>CO₂</th>
<th>Lipid</th>
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<tbody>
<tr>
<td>Fasted</td>
<td>-0.8 ± 0.7 I 5.5 ± 1 I 0.26 ± 0.19</td>
<td>-0.8 ± 0.7</td>
<td>-0.8 ± 0.7</td>
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<td>0.2</td>
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<tr>
<td>+α-ketobutyrate</td>
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<td>+1.3 ± 1.9</td>
<td>+1.3 ± 1.9</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>+α-ketobutyrate</td>
<td>+1.5 ± 1.8 I 9.6 ± 1.7</td>
<td>+1.5 ± 1.8</td>
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<td>31</td>
<td>11</td>
</tr>
<tr>
<td>+α-ketobutyrate</td>
<td>+1.7 ± 1.9 I 4.6 ± 1.5</td>
<td>+1.7 ± 1.9</td>
<td>+1.7 ± 1.9</td>
<td>25</td>
<td>14</td>
</tr>
</tbody>
</table>
also substantial inhibition of pyruvate oxidation by hydroxy-
pyruvate, and to a lesser extent by α-ketobutyrate.

The stimulation by α-ketobutyrate of L-lactate metabolism by
adipose tissue of fasted rats is shown in Table V. The incorpora-
tion of L-lactate-U,14C into CO2 and lipid was stimulated, and
there was also net L-lactate utilization. Table VI shows utiliza-
tion of L-lactate and α-ketobutyrate, added together at equal
concentrations to the fat pad segments of fasted-refed and fasted
rats. In refed rats α-ketobutyrate depressed L-lactate utiliza-
tion, and pyruvate and α-hydroxybutyrate accumulated. We
found that added hydroxybutyrate is only slightly utilized by
adipose tissue. α-Ketobutyrate is also metabolized by other
pathways, most probably by decarboxylation to propionyl-CoA.
The stoichiometry between L-lactate uptake and formation of
hydroxybutyrate indicates that in tissue of fasted rats most of
the stimulation of L-lactate uptake may be considered a form of
hydrogen exchange, catalyzed by lactate dehydrogenase. There
is no transfer of hydrogen equivalents into mitochondria, and no
oxidation of this hydrogen to H2O.

It was of interest to see what effect a supply of an exogenous
hydrogen acceptor for mitochondrial DPNH would have on L-
lactate utilization by adipose tissue of fasted rats. β-Hydroxy-
butyrate dehydrogenase is a tightly bound mitochondrial enzyme,
and it is reported to be present in adipose tissue (18), and hence
we studied the effects of acetocetate. Addition of acetocetate
increased incorporation of 14C from L-lactate into CO2 and fatty
acids and also caused net utilization of L-lactate (Table VI).
β-Hydroxybutyrate and acetate additions were without effect.

<table>
<thead>
<tr>
<th>Substrate and label</th>
<th>Lactate utilization</th>
<th>14C in μatoms/g/3 hr</th>
<th>Lipid μatoms/g/3 hr a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniformly labeled; three experiments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Lactate-U,14C</td>
<td>1.9 ± 0.2</td>
<td>6.9 ± 0.4</td>
<td>0.4 ± 0.16</td>
</tr>
<tr>
<td>L-Lactate-U,14C</td>
<td>6.3 ± 0.0</td>
<td>16.7 ± 0.0</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>acetocetate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specifically labeled; one experiment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Lactate-2,14C</td>
<td></td>
<td>%b</td>
<td>%b</td>
</tr>
<tr>
<td>L-Lactate-2,14C</td>
<td>7.0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>acetocetate</td>
<td>8.3</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Acetocetate-3,14C</td>
<td>16.4</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>L-Lactate + acetocetate-3,14C</td>
<td>19.5</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>L-Lactate-2,14C + acetate</td>
<td>6.6</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>L-Lactate + acetate-1,14C</td>
<td>12.7</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

*a This represents the conversion of L-lactate-U,14C to CO2 and
lipid.
*b The results represent the percentage of added 14C in CO2 and
lipid.

However, the interpretation of the mechanism of acetocetate
stimulation is difficult. Hanson and Ziporin (16) reported ap-
preciable oxidation of labeled acetocetate and incorporation of
this compound into lipids by adipose tissue. The metabolism of
β-hydroxybutyrate was much lower, about one-tenth that of
acetocetate. Our results with acetocetate-3,14C (Table VII)
confirm those of Hanson and Ziporin, and we also found the
utilization of tritium-labeled β-hydroxybutyrate to be quite low.
Thus the stimulation of L-lactate metabolism may be coupled to
the reduction of the cytoplasmic acetocetate-CoA, rather than
reduction of acetocetate in the mitochondria. It is obscure
why, in the presence of L-lactate, adipose tissue of fasted rats in-
corporates acetocetate but not acetate and β-hydroxybutyrate
into fatty acids, and further studies are required.

**TABLE VII**

Effect of acetocetate on L-lactate utilization by adipose tissue of
fasted rats

Epididymal fat pad tissue (250 mg) was incubated in 1 ml of
Krebs-Henseleit bicarbonate buffer under the conditions of
Table I. Concentration of L-lactate, acetocetate, and acetate
5 mm.

**DISCUSSION**

Supply of Reducing Equivalents for Fatty Acid Synthesis—Un-
der optimal conditions, rat adipose tissue can utilize glucose,
L-lactate, and pyruvate as substrates for fatty acid synthesis.
The major difference in lipogenesis from these compounds is the
source of hydrogen equivalents to reduce acetyl-CoA to fat.
Under lipogenic conditions (fat-free diet, refeeding after a fast)
and with glucose as substrate, the hydrogen equivalents are de-
\riage via the pentose cycle and the oxidation of glyceraldehyde-P
to phosphotiglyceric acid (9, 10). With L-lactate as substrate,
a little more than half of the reducing equivalents may be provided
by oxidation of L-lactate to pyruvate, but the rest must be derived
from the mitochondria from the oxidation of pyruvate. With
pyruvate as substrate, all the reducing equivalents must be de-
\irived from mitochondrial oxidation of pyruvate.

Reducive biosynthesis of fatty acids in adipose tissue is a cy-
toplasmic process. When hydrogen equivalents are produced in
mitochondria, these must be transferred into cytoplasm. Malate
is generally held to be the carrier, providing TPNH by decar-
boxylation catalyzed via the malic enzyme. Irrespective of the
mechanism, this transfer requires energy, provided by mito-
chondrial oxidation. It follows that in lipogenesis from pyruvate
a larger fraction of the substrate carbon must be oxidized to pro-
\vide the required extra hydrogen equivalents for reduction, as
well as additional energy, than if L-lactate is the substrate.

Hence pyruvate is theoretically a less efficient substrate for fatty
acid synthesis than L-lactate. Our results fully support this con-
clusion. Whereas under optimal conditions for lipogenesis about
equal amounts of L-lactate and pyruvate are utilized (Table I),
50 to 55% of L-lactate carbons are converted to fatty acids and
about 45% to CO2 (Table II). Under the same conditions, only
33 to 38% of pyruvate carbons are converted to fatty acids and
about 55% to CO2. A more complete analysis of energy balance
in fatty acid synthesis from these two acids will be presented else-
where.

Oxidation of Cytoplasmic DPNH and Metabolism of L-Lactate—
Under lipolytic conditions induced by either fasting or lipolytic
hormones, fatty acid synthesis from glucose is impaired and
oxidation via the Krebs cycle is increased (9, 10). This is true
also of pyruvate (Table II), and the incorporation of pyruvate
into glyceroal equals or exceeds that in fatty acids. Under these
conditions, the formation of cytoplasmic hydrogen equivalents
from L-lactate via the lactate dehydrogenase reaction would
greatly exceed their utilization in lipogenesis. Hence if no
mechanism for oxidation of cytoplasmic DPNH is available,
metabolism of L-lactate will be severely inhibited.
We have previously shown (17) that when 2,4-dinitrophenol is added to adipose tissue from fed rats, lipid synthesis is practically abolished, but oxidation of pyruvate is not impaired. Under these conditions, however, L-lactate oxidation was greatly depressed. We have interpreted this inhibition as due to the inability of adipose tissue to regenerate the cytoplasmic DPNH. This explanation fits well our findings on L-lactate metabolism under lipolytic conditions, induced either by fasting or epinephrine.

In the metabolism of glucose under lipolytic conditions, the oxidation of glucose carbon is also limited by the oxidation of cytoplasmic DPNH. Glucose under these conditions can, of course, be converted to L-lactate. However, for each pyruvate molecule entering mitochondria, a molecule of DPNH must be reoxidized. With glucose, dihydroxyacetone-P may serve as a hydrogen acceptor. Thus, glucose metabolism (neglecting L-lactate formation) under lipolytic conditions represents approximately a dismutation to 1 molecule of pyruvate and 1 molecule of ATP-glycerophosphate. Our previous results are consistent with this stoichiometry. Thus (10) in the presence of epinephrine the specific yield from glucose-U-14C in lipid glycerol and soluble glycerol (released into the medium) was 34%, nearly identical with the yield of 14CO2. 

Generation of cytoplasmic oxaloacetate from aspartate plus a-ketoglutarate stimulated L-lactate utilization and oxidation, and restored lipogenesis. The effect was probably due to the regeneration of DPN from DPNH in cytoplasm, permitting the formation of pyruvate from L-lactate. The failure of stimulation by a-ketoglutarate by itself suggest that the mitochondrial membranes are not freely permeable to oxaloacetate generated in mitochondria, and that a transaminase shuttle for oxaloacetate transfer, such as suggested for liver by Lardy et al. (18) does not operate, at least in adipose tissue of fasted rats.

We considered the possibility that the inability to oxidize cytoplasmic reducing equivalents is due to a large difference in oxidation-reduction potential between mitochondria and cytoplasm (see below), and attempted to lower the oxidation-reduction potential in mitochondria by the reduction of acetoacetate. Addition of acetoacetate caused a stimulation of L-lactate utilization and lipogenesis. There was no appreciable formation of 2-hydroxybutyrate, but incorporation of oxaloacetate into lipid. The mechanism of acetoacetate effect is not clear, and the oxidation of L-lactate to pyruvate may be coupled with cytoplasmic acetoacetyl-CoA reduction.

A shuttle for transport of hydrogen equivalents from cytoplasm to mitochondria was proposed by Bücher and Klingenberg (19) and Estabrook and Sacktor (20). It involves reduction of dihydroxyacetone phosphate to a-glycerophosphate by cytoplasmic DPNH, and reoxidation of a-glycerophosphate in mitochondria. The mitochondrial reaction is catalyzed by a membrane-bound flavin enzyme. The net over-all reaction is thus reduction of mitochondrial flavin by cytoplasmic DPNH. Bücher and Klingenberg (19) pointed out that the reaction involves a large decrease in free energy and is thus irreversible. The a-glycerophosphate shuttle is a major pathway in insect flight muscle (19, 20) and probably operates in some mammalian tissues.

Adipose tissue contains, in addition to a very active cytoplasmic a-glycerophosphate dehydrogenase, the mitochondrial enzyme (21). Lee and Lardy (21) have shown that the level of this enzyme is greatly increased in hyperthyroid rats. Isolated mitochondria of adipose tissue were found to oxidize added a-glycerophosphate at high rates (22). It is surprising that in the presence of the enzyme, the shuttle does not seem to operate. The effect of fasting on this enzyme is not known, and it may be depressed or inhibited. However, in the presence of dinitrophenol the shuttle does not seem to operate in tissues of fed and even hyperthyroid rats (17).

The inability to transfer hydrogen equivalents from cytoplasm to mitochondria of adipose tissue may be due to a very high energy barrier. In liver the DPNH:DPN+ ratio in mitochondria is much greater than in cytoplasm (23, 24), and this is probably true of all tissues. If the oxidation-reduction potential in mitochondria is higher than in the cytoplasm, energy will be required for the transfer of hydrogen equivalents. It is conceivable that the oxidation-reduction gradient could become so large that in spite of the favorable energy of the a-glycerophosphate system, the transfer of hydrogen equivalents into mitochondria becomes thermodynamically highly unfavorable. The energy required to transfer a mole of hydrogen equivalents against an oxidation-reduction gradient can be approximated by 2.3RT log K. K is the ratio

\[
\frac{[DPNH_2]}{[DPN^+]}, \frac{[DPN^+]}{[DPNH]} \]

where m and c refer to mitochondrial and cytoplasmic adenine nucleotides, and at 37°C the value of 2.3RT is 1.4 kcal. If K is 10,000, the minimal energy for hydrogen transfer would be 4 × 1.4 = 5.6 kcal, and for hydrogen transfer into mitochondria to proceed at appreciable rates, excess energy will be required. The decrease in free energy in the reduction of a flavin with DPNH is roughly equal to that available from one high energy phosphate bond, about 7 kcal. It follows that the shuttle will not operate efficiently when the difference in DPNH:DPN+ ratios is over 10,000. In liver ratios of the order of hundreds were measured (23). No comparable data exist for adipose tissue. It is questionable whether ratios of the order of 10,000 can be obtained. The reason for the nonfunctioning of the a-glycerophosphate shuttle requires further study.

Our studies on L-lactate metabolism show that in adipose tissue transfer of hydrogen equivalents occurs only from mitochondria to cytoplasm, but not in the reverse direction. It confirms our previous conclusion that an a-glycerophosphate shuttle does not operate in this tissue. Other tissues such as brain and muscle...
oxidize lactate, with CO₂ as the major product, and hence contain a shuttle or some other mechanism to reoxidize cytoplasmic DPNH to DPN. It remains to be seen whether the absence of a hydrogen shuttle is a unique property of rat adipose tissue.

A balance of hydrogen equivalents in glucose metabolism of adipose tissue has been determined by Flatt and Ball [27] and by ourselves [28, 29]. It was found [27, 29] that addition of acetate increases lipogenesis and the utilization of glucose. Flatt and Ball [27] have suggested that oxidation of “excess” reducing equivalents may be a limiting factor in lipogenesis. Our results stress the close integration between hydrogen equivalent balance and metabolic control. Our study suggests that in adipose and possibly other tissues the transport of hydrogen equivalents between compartments may be an important factor in the control of biosynthesis and metabolism.

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