The Metabolism of Pyruvate in the Tricarboxylic Acid Cycle of Developing Mammalian Liver*

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Previous work has indicated that much of the biochemical maturation of the mammalian liver takes place around the time of birth (1–6). In the present study of this critical period, we have investigated the mode of pyruvate utilization in the tricarboxylic acid cycle. Pyruvate enters the tricarboxylic acid cycle in two ways, as acetyl coenzyme A and as a dicarboxylic acid. In the following experiments, the apportionment of pyruvate entering the tricarboxylic acid cycle between acetyl coenzyme A and dicarboxylic acid was estimated in vivo by determining the relative distribution of isotope in glutamic acid after injection of \( \text{DL-alanine-2-C}^{14} \). The method is based on the observation that the position of isotopic labeling in glutamic acid by pyruvate-2-C\(^{14} \) depends on the route of entry of pyruvate into the tricarboxylic acid cycle (7, 8).

The mode of pyruvate utilization in the tricarboxylic acid cycle was studied at various stages of development in guinea pig liver. The stages were chosen to coincide with different phases in the metabolism of glycogen and fat (9, 10). In guinea pig liver, the functional state of the glycogen and fat stores around the time of birth may be divided into three phases. First, before the 57th day of gestation, glycogen is present only in traces, and the fat concentration is stabilized at about twice the level of the maternal liver; second, from the 57th day until birth (68 days), both the glycogen and fat concentrations gradually increase to values 3 and 4 times those found in the mother; and third, during the first few days after birth, glycogen and fat are rapidly depleted. The apportionment of pyruvate in the tricarboxylic acid cycle between acetyl coenzyme A and dicarboxylic acid was studied in 57- and 68-day-old fetuses, when the fetuses are at the beginning and the height of glycogen and fat storage; in 4-hour-old newborns when the rate of fat and glycogen depletion is highest; at 30 hours after birth when the fat concentration is still falling rapidly; at 10 days after birth when the fat concentration is still falling but at a very reduced rate; and at 30 days after birth when the fat and glycogen concentrations have stabilized at normal adult values.

**EXPERIMENTAL PROCEDURE**

After injection of pyruvate-2-C\(^{14} \) or \( \text{DL-alanine-2-C}^{14} \) into an animal, the relative isotope distribution in glutamic acid depends on the mode of entrance of pyruvate into the tricarboxylic acid cycle. Isotope is distributed to carbon 5 of glutamic acid by pyruvate-2-C\(^{14} \), entering the cycle as acetyl-CoA-1-\(^{14} \). Carbon atoms 2 and 3 are labeled by pyruvate-2-C\(^{14} \), entering the cycle as a dicarboxylic acid. Carbon 1 is labeled in both cases by recycling and can be ignored when making an estimate of the apportionment of pyruvate between acetyl-CoA and dicarboxylic acid. The maximal percentage of isotope that would be found in carbon 5 under conditions in which all pyruvate enters the cycle as acetyl-CoA is 67%, the remainder would be in carbon 1 (7).

An interpretation of the isotope distribution in glutamic acid must consider not only the apportionment of pyruvate between acetyl-CoA and dicarboxylic acid, but also the differential dilution of these two compounds by unlabeled sources. It is perhaps reasonable to assume in our experiments with well-fed animals that pyruvate is the major source of dicarboxylic acid in the tricarboxylic acid cycle. However, the acetyl-CoA labeling in glutamic acid is certainly diluted by acetyl CoA derived from fatty acids (7). The labeling of carbon 5 by pyruvate-2-C\(^{14} \), therefore, is the result of two processes; the apportionment of pyruvate and fatty acid oxidation.

In the present study two pregnant guinea pigs were given intraperitoneal injections of \( \text{DL-alanine-2-C}^{14} \) (100 \( \mu \)g per kg of body weight), one on the 44th day of the gestational period, the other on the 68th day (term). The animals were killed by cervical dislocation 1 hour after injection. The maternal livers, fetal livers, and placentas were excised and hydrolyzed in HCl. The placenta from a litter were pooled as were the fetal livers. Glutamic acid was isolated from the hydrolysates and degraded. The isotopic content of each carbon atom was measured (7).

Similarly, \( \text{DL-alanine-2-C}^{14} \) was injected intraperitoneally (300 \( \mu \)g per kg of body weight) into four littersmates. One animal was treated at the age of 4 hours, another at 30 hours, another at 10 days, and the last at 30 days. One hour after injection, these animals were killed by decapitation; livers were excised and hydrolyzed in HCl. The glutamic acid was isolated and analyzed as before (7).

Guinea pigs were obtained from our inbred colony maintained on ad libitum feedings of pellets (protein, 18%; fat, 3%; carbohydrate, 62%) fortified with ascorbic acid and supplemented with a daily ration of fresh green vegetables. All animals used in these experiments were well fed and had eaten within the hour before the injection of isotopically labeled \( \text{DL-alanine} \). Their stomachs were full of ingested material at the time of death. The stomachs of the 4- and 30-hour-old animals contained milk obtained by nursing. The 10-day-old animal subsisted largely on pellets and green vegetables. The 30-day-old animal had been weaned at 20 days of age.
Fetal age was determined on the basis of crown to rump length and weight and reference to Drapers' table (11). DL-Alanine-2-C\(^{14}\) was purchased from Isotope Specialties, Inc.

**RESULTS**

The distribution of isotope in glutamic acid isolated from livers at various stages of development is given in Table I. In fetal liver, the distribution of isotope indicates that the apportionment of pyruvate in the tricarboxylic acid cycle between acetyl-CoA and dicarboxylic acid is predominantly to acetyl-CoA and is about the same at the beginning (57 days) and at the height (68 days) of glycogen and fat storage. Carbon 5 contained over 50% of the radioactive label and carbon atoms 2 and 3 less than 30% at both of these fetal stages.

In newborn liver, the isotope distribution indicates that there is a rapid change at birth in the apportionment of the pyruvate entering the cycle toward dicarboxylic acid formation. The shift is not a simple distribution but is a matter of conjecture. There is a rapid rise in the percentage of the radioactive label in carbon 5 and the percentage of carbon atoms 2 and 3 less than 10% difference among individuals.

<table>
<thead>
<tr>
<th>Source of glutamic acid</th>
<th>Radioactivity in each carbon atom</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal liver, 57 days*</td>
<td>% % % % % % % % % % % % % % % % % % % % % % % % % %</td>
<td></td>
</tr>
<tr>
<td>Fetal liver, 68 days (term)*</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Liver of postnatal stages†</td>
<td>4 hours</td>
<td>10 30 35 2 20 97</td>
</tr>
<tr>
<td></td>
<td>30 hours</td>
<td>9 33 48 0.5 7 98</td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>12 26 30 1 21 96</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>14 8 13 2 60 97</td>
</tr>
<tr>
<td>Maternal liver, 57 days</td>
<td>18 11 17 1 55 99</td>
<td></td>
</tr>
<tr>
<td>Maternal liver, 68 days (term)</td>
<td>20</td>
<td>44</td>
</tr>
<tr>
<td>Placenta, 57 days*</td>
<td>20 14 7 2 54 97</td>
<td></td>
</tr>
<tr>
<td>Placenta, 68 days (term)*</td>
<td>21</td>
<td>59</td>
</tr>
</tbody>
</table>

* Pooled organs of littermates.
† Each stage is a single animal. The four postnatal stages are littermates.

**DISCUSSION**

Previous work has suggested that fasting causes a shift in the apportionment of pyruvate in the tricarboxylic acid cycle of fetal liver (1-6). Fasting experiments with rats indicated that the rate of dicarboxylic acid formation from pyruvate in the fetal period is proportional to the rate of fatty acid oxidation in the newborn. This shift in the mode of pyruvate utilization in the tricarboxylic acid cycle is one of many biochemical changes that occur in mammalian liver during the transition from fetal to extrauterine life (1-6).

The mechanisms that control the apportionment of pyruvate in the tricarboxylic acid cycle are a matter of conjecture. There are a number of enzymes in adult liver that can carry out the carboxylation of pyruvate to a four carbon compound. Absence of liver enzyme activity in fetal liver with a rapid rise in activity in the newborn could explain the shift to dicarboxylic acid formation at birth. A low cofactor concentration for any of the necessary enzymes could also limit dicarboxylic acid formation. As expected, the specific activities of carbon 5 in the postnatal stages do not change reciprocally in any exact way with the specific activities in carbon atoms 2 and 3. This is so presumably because the acetyl-CoA derived from pyruvate and entering the tricarboxylic acid cycle is diluted unequally at the various stages by unlabeled acetyl-CoA from fatty acids. The dilution seems to be most marked at the age of 30 hours when, indeed, the rate of fatty acid oxidation is highest (9).
towards dicarboxylic acid formation at birth and in the development of a mechanism to regulate the mode of entrance of pyruvate into the tricarboxylic acid cycle.

Another mechanism depending on the concentration of a cofactor is suggested by the pyruvate carboxylating system described by Utter and Kechoh (13). This enzyme requires acetyl-CoA as a cofactor. Perhaps in the newborn a rise in the acetyl-CoA concentration resulting from fat breakdown increases the activity of this system. In our experiments, the rate of dicarboxylic acid formation from pyruvate seemed to be proportional to the rate of fatty acid oxidation (Table II). A regulatory mechanism in which pyruvate carboxylation is controlled by the acetyl-CoA concentration is attractive because it links the availability of dicarboxylic acids to the rate of acetyl-CoA formation from fat.

SUMMARY

1. The apportionment of pyruvate entering the tricarboxylic acid cycle between acetyl coenzyme A and dicarboxylic acid was estimated at different stages of development in guinea pig liver in vivo. In fetal and adult liver, pyruvate was found to enter the tricarboxylic acid cycle largely as acetyl coenzyme A, in the newborn largely as a dicarboxylic acid.

2. In the newborn period, the rate of dicarboxylic acid formation from pyruvate in liver seemed to be proportional to the rate of fatty acid oxidation.

REFERENCES

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