

THE OXIDATION OF CHOLINE BY LIVER SLICES AND MITOCHONDRIA
DURING LIVER DEVELOPMENT IN THE RAT

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Summary

Betaine is the major oxidation product of [Me-¹⁴C] choline produced by rat liver slices. Liver slices from adult rats rapidly oxidize [Me-¹⁴C] choline to betaine and the bulk of the betaine produced is recovered in the incubation medium. Considerably more choline is oxidized to betaine than is phosphorylated to phosphorylcholine. The rate of phosphorylation of choline appears to be independent of the rate of choline oxidation. Liver slices from fetal and young rats oxidize choline to betaine at a lower rate than adult liver slices.

The ability of mitochondria to oxidize [Me-¹⁴C] choline to betaine aldehyde and betaine is considerably lower in fetal liver than in adult liver. The major product with both fetal and adult mitochondria is betaine aldehyde. Choline oxidation by mitochondria begins to increase 1 day prior to birth and increases progressively to adult levels by 18 days. The developmental pattern for choline oxidation is similar to the pattern for succinic dehydrogenase activity.

Choline can be metabolized by the liver through two major pathways. One pathway is initiated by the enzyme choline kinase (ATP: choline phosphotransferase, E.C. 2.7.1.32) and subsequently leads to the synthesis of choline phosphoglycerides (1). The other pathway involves the oxidation of choline to betaine, which subsequently donates its methyl group to homocysteine to form methionine (2). According to Wong and Thompson (3) the pool of free choline in the liver is much smaller than that of betaine and phosphorylcholine, indicating that dietary choline is efficiently converted to betaine and phosphorylcholine. The relatively high concentrations of betaine and phosphorylcholine found in the

liver suggest that these may be storage forms of methyl groups from choline for subsequent metabolic use in their respective pathways. The mechanisms that regulate the relative involvement of the pathways are largely unknown.

We have observed previously that the concentration of phosphorylcholine is considerably higher in fetal liver than in adult liver and the drop in concentration during development generally correlates with the rise in the rate of choline phosphoglyceride synthesis (4). In these same experiments we found that liver slices rapidly converted the choline in the incubation medium to a water-soluble metabolite. The present paper identifies this metabolite as betaine and shows that the ability of both liver slices and isolated mitochondria to oxidize choline to betaine aldehyde and betaine is significantly lower in fetal liver than in adult rat liver.

Materials and Methods

Pregnant rats of specified delivery dates (+ 12h) and female rats (200-220 gm) were purchased from Holtzman Co., Madison Wisconsin. The animals were given Purina Rat Chow for at least 1 week prior to the experiments. [Me-¹⁴C] choline was obtained from New England Nuclear Corporation.

Liver slices were prepared with a Stadie-Riggs hand microtome. Slices (200-250 mg) were incubated, as previously described (5), in an atmosphere of 95% O₂ - 5% CO₂ in 2.0 ml of Krebs-bicarbonate medium (pH 7.4). The incubation medium contained 2 mg/ml glucose and 1.3 mM [Me-¹⁴C] choline (Sp. Act. 0.77 μ Ci/ μ mole). After the desired incubation the slices plus the medium were homogenized in 20 volumes of 2:1 chloroform-methanol, followed by the addition of 0.2 volume of H₂O. The solvent layers were separated by brief centrifugation and the methanol-water layer removed and evaporated to dryness in a vacuum evaporator. The residue was dissolved in a small amount of water and analyzed by paper chromatography. Phosphorylcholine was separated by two-dimensional paper chromatography as described previously (6). Betaine was routinely separated from choline and betaine aldehyde by paper chromatography with the solvent system 95% ethanol-concd NH₄OH, 95:5. Radioactivity was detected by the chro-

matogram by autoradiography and the radioactive areas cut from the chromatogram. The radioactivity was quantitatively determined in a liquid scintillation counter after placing the paper sections directly into scintillation vials that contained toluene scintillation fluid. Nonradioactive standards were detected with reagents described by Cromwell and Richardson (7).

Mitochondria were isolated from a 10% homogenate in 0.30 M sucrose. The homogenate was centrifuged at 600g for 10 min. The 600g supernatant was centrifuged at 20,000g for 10 min. The resulting mitochondrial pellet was washed twice by suspending the pellet in 0.30 M sucrose and centrifuging at 20,000g for 10 min. The washed mitochondria were suspended in 0.30 M sucrose; 1.0 ml of sucrose per gram of liver.

The oxidation by mitochondria of [Me-¹⁴C] choline to betaine aldehyde and betaine was determined by a slight modification of a method described by Wilken (8). The incubation mixture contained Tris buffer pH 7.5, 5 μ moles; potassium phosphate buffer, pH 7.5, 3 μ moles; MgSO₄, 1.5 μ moles; NAD, 0.3 μ mole; [Me-¹⁴C] choline 3.0 μ moles (Sp. Act. 0.05 μ Ci/ μ mole) and mitochondria from 100 mg of liver. The final volume of the reaction was 0.3 ml. The reaction mixture was incubated for 40 min. at 37°C. The reaction was stopped by adding 0.3 ml of methanol. A portion of this mixture was applied to silica gel TLC plates and chromatographed in methanol; acetone; concd HCl; 90:10:4. The radioactivity was detected by autoradiography. The amount of radioactivity in betaine aldehyde and betaine was determined by scraping those areas of TLC plate into scintillation vials and counting them in toluene scintillation fluid in a liquid scintillation spectrometer.

Succinic dehydrogenase was determined as described by Slater and Planterose (9). Protein was measured by the Lowry method (10).

Results

[Me-¹⁴C] Choline Oxidation by Liver Slices

The production of radioactive betaine from [Me-¹⁴C] choline by liver slices from adult rats is shown in Table I. Betaine is rapidly produced by the slice

TABLE I

The Oxidation of [Me-¹⁴C] Choline to Betaine by Liver
Slices from Adult Female Rats. Each Value is the Average of
Duplicate Incubations

Incubation Length (min)	Betaine Production (μ mole/gm Liver)		
	Media	Slice	Total
15	3.00	1.10	4.20
30	5.80	2.00	7.30
60	6.85	2.65	9.50
120	7.05	3.80	11.25

and relatively large amounts are transported into the medium. At the end of a 2h incubation only trace amounts of radioactive choline were detected. In these incubations 200 mg of liver slices were incubated in 2.0 ml medium that contained a total of 2.6 μ moles of choline. After 2h of incubation 2.3 μ moles of betaine was obtained and most of the remainder of the choline was converted to phosphorylcholine.

Similar incubations were performed with slices from rats at -2 day and 7 day. The maximum formation of betaine from [Me-¹⁴C] choline is lower with fetal and young than with adult, (Fig. 1). Interestingly, the initial rate of formation of betaine by the slices is about the same in fetal as in adult. The total flow of choline through the choline kinase reactions was estimated by adding the amount of radioactivity recovered in phosphorylcholine to the amount recovered in choline phosphoglycerides. This sum does not change appreciably during these stages of development. However, as reported previously (4), a greater percentage of the radioactivity is found in phosphorylcholine in slices from fetal than in slices from young and adult. No radioactive betaine

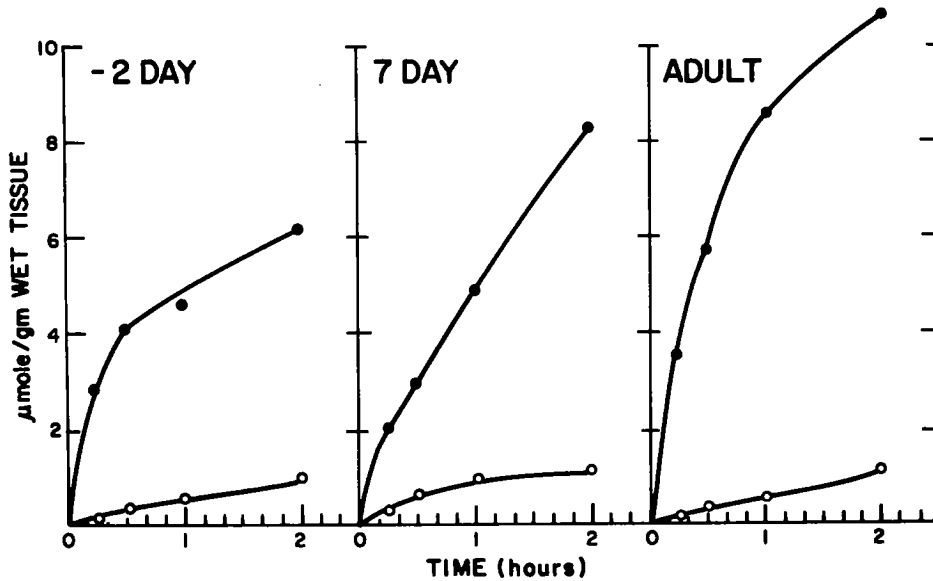


FIG. 1

The oxidation of choline to betaine and the phosphorylation of choline to phosphorylcholine by liver slices from fetal, young and adult rats. Each point is the average of duplicate incubations. ●—●, choline to betaine; ○—○, choline to phosphorylcholine plus choline phosphoglycerides.

aldehyde was detected in any slice experiments. Thus the decline in the rate of formation of betaine by fetal slices apparently results from a decrease in the initial oxidation of choline.

Production of Betaine Aldehyde and Betaine by Mitochondria

Mitochondria from -4 day rat liver produce considerably less betaine aldehyde from choline than mitochondria from adult rat liver, Table II. Both betaine aldehyde and betaine were produced. Betaine aldehyde accounted for 80-82% of the radioactive products, a result consistent with the observations of Wilken, et. al. (8). The relative amounts of betaine aldehyde and betaine were the same with both fetal and adult mitochondria.

The ability of mitochondria to oxidize choline to betaine aldehyde plus betaine was measured at various developmental ages. Succinic dehydrogenase

TABLE II
 Production of Betaine Aldehyde and Betaine from Choline by
 Mitochondria from Adult and -4 Day Fetal Liver.

Age	Betaine Aldehyde (nMoles/min/mg Protein)	Betaine
Adult	16.0	3.5
	15.4	2.1
-4 day fetal	3.1	0.6
	3.3	0.9

activity was determined on the same preparations. Choline oxidation when expressed per mg of fresh liver was 10% of the adult values at -4 days and -2 days, (Fig. 2A). The activity began to increase at -1 day and increased progressively to adult levels by 18 days. Succinic dehydrogenase activity followed the same general pattern but had a higher activity relative to the adult value. When the activities were expressed as units per mg of mitochondrial protein, a developmental pattern was observed that was in general similar to that obtained on a tissue weight basis, (Fig. 2B).

Discussion

Our results show that betaine is the predominant product when choline is metabolized by rat liver slices. Much greater amounts of choline are oxidized to betaine than are phosphorylated to phosphorylcholine. Sung and Johnstone (11) obtained a similar result with rat kidney slices. They observed that about 80% of the radioactivity in kidney slices after incubation with [Me-¹⁴C] choline was in the form of betaine. They also reported that (¹⁴C)-betaine was not metabolized significantly by the kidney slice preparations. In our experiments very little if any betaine was metabolized further. This may be caused by a lack of homocysteine as a methyl acceptor in the slice incubation. Studies in the intact rat by Wong and Thompson indicate that

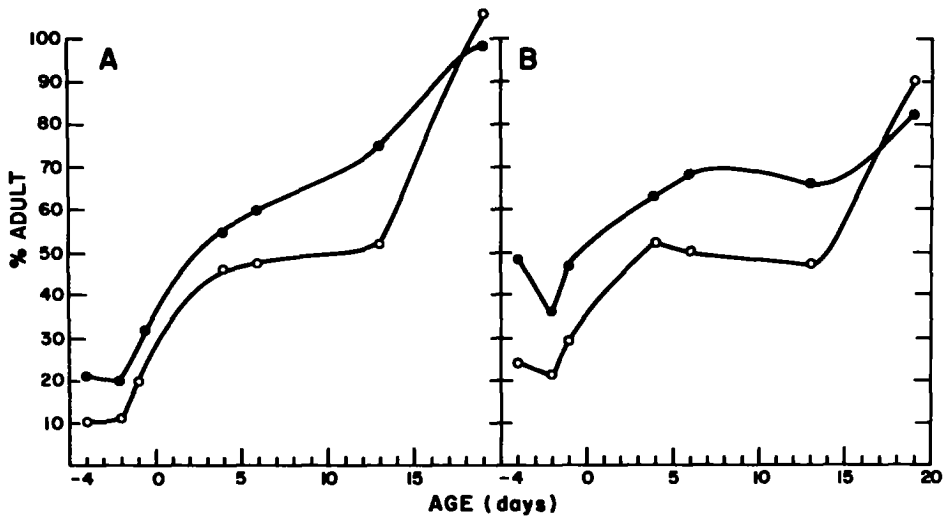


FIG. 2

The oxidation of choline by liver mitochondria. Each point is the average of triplicate assays, ●, succinic dehydrogenase activity; ○, choline oxidation; A, expressed on a wet liver basis; B, expressed on a mitochondrial protein basis. Adult values are: choline oxidation, 0.69 $\mu\text{mole}/\text{min}/\text{gm}$ tissue and 16 nmoles/min/mg protein, succinate-neotetrazolium reductase, 0.45 μmole formazan produced/min/mg tissue and 0.011 μmole formazan produced/min/mg protein.

choline is efficiently converted to betaine by the liver and the betaine concentration is relatively high. Thus, the transfer of the methyl groups of betaine to form methionine may be considerably slower than the formation of betaine and our results with liver slices may also be a reflection of this relationship.

The ability of mitochondria from fetal liver to oxidize choline to betaine aldehyde is about 10% of the adult whereas the oxidation of choline to betaine by fetal liver slices is about 70% of the adult. Furthermore, the oxidation of choline by adult mitochondria is greater than the oxidation of choline to betaine by adult liver slices (0.69 $\mu\text{mole}/\text{min}/\text{gm}$ tissue vs 0.38 $\mu\text{mole}/\text{min}/\text{gm}$ tissue), while, in -2 day fetal the oxidation by slices is greater than the oxidation by mitochondria (0.27 $\mu\text{mole}/\text{min}/\text{gm}$ tissue vs

0.08 $\mu\text{mole}/\text{min}/\text{gm}$ tissue). The oxidation of choline to betaine by liver slices involves both the oxidation of choline to betaine aldehyde and the further oxidation of betaine aldehyde to betaine. The latter reaction is located predominantly in the soluble portion of the cell (12, 13). The exact interplay between these two reactions for the complete oxidation of choline to betaine is unclear. However, it appears that the capacity of mitochondria to oxidize choline does not solely establish the total rate of choline oxidation to betaine by intact cells.

The phosphorylation of choline by liver slices appears to be relatively independent of the oxidation of choline. Even when 80% of the choline has been oxidized to betaine, the rate of choline phosphorylation continues relatively unchanged. This is particularly apparent in the adult experiment, Fig 1. This suggests that choline kinase in the hepatocyte can efficiently phosphorylate choline as it enters the cell. This is consistent with the observation by Wong and Thompson that the choline pool in the liver is small compared to the pool of phosphorylcholine.

The developmental pattern for choline oxidation by isolated mitochondria is very similar to that obtained for succinic dehydrogenase activity. The present developmental pattern for succinic dehydrogenase is essentially the same as previously reported (14, 17). Several studies have indicated that the total activity of respiratory enzymes increases after birth (15, 17). The developmental pattern for choline oxidation coincides with this general pattern for respiratory enzymes. Our results are also in complete agreement with the conclusions presented by others (15,17,19) that mitochondria undergo qualitative changes at various stages of development.

Acknowledgements

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