CONCERNING THE FORM IN WHICH ACETYL UNITS PRODUCED IN MITOCHONDRIA ARE TRANSFERRED TO THE SITE OF DE NOVO FATTY ACID SYNTHESIS IN THE CELL*

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It has been demonstrated that enzymes involved in conversion of glucose-6-phosphate to pyruvate (1) and in de novo‡ formation of fatty acids (2-5) are located mainly in cell sap, whereas those concerned with conversion of pyruvate to acetyl-CoA are in the mitochondria (1,6). This raises the interesting question of how acetyl-CoA, or a derivative of it, is transported from the mitochondria to the site in the cell where de novo fatty acid synthesis takes place. Four possibilities have been suggested (7,8): (a) Acetyl-CoA diffuses as such across the mitochondrial membrane (9); (b) Acetyl-CoA is first hydrolyzed in the mitochondria, and the acetate formed there is then transported to cell sap where it is reconverted to acetyl-CoA (10); (c) Acetyl-CoA is converted by mitochondrial transacetylase

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‡ The term de novo as used here refers to synthesis from acetyl-CoA by the pathway involving the intermediate formation of malonyl-CoA.

to acetylcarnitine (11) which is transported to cell sap and reconverted there to acetyl-CoA;* (d) Acetyl-CoA is converted by the condensing enzyme in the mitochondria to citrate. In this last scheme, the acetyl unit is transported to the extramitochondrial compartment either as citrate (13,14) or α-ketoglutarate or glutamate (15) or as all three or any two; these, as the case may be, are then converted to acetyl-CoA by enzymes in the extramitochondrial part of the cell so that the acetyl unit attached to oxalacetate by the mitochondrial condensing enzyme is the same unit as that released by the cytoplasmic citrate-cleavage enzyme (14-16).

In none of these four schemes would carbon originating from the methyl position of pyruvate be removed from acetyl-CoA, and only in scheme (d), where the acetyl-CoA formed from pyruvate condenses with oxalacetate yielding citrate in the mitochondria, would hydrogen from the methyl group of acetyl-CoA be removed. Since the two terminal carbons at the methyl end of a fatty acid chain synthesized de novo are derived from acetyl-CoA without loss of methyl-bound hydrogen (17), a comparison of the incorporation of the methyl carbon of pyruvate with that of the methyl hydrogen of pyruvate into the terminal methyl end of the fatty acid chain should tell us whether citrate is an intermediate in fatty acid synthesis. The experiments described here were designed to test this point.

We studied the distribution of ^{3}H and ^{14}C in the fatty acids synthesized by slices of lactating rat mammary glands

* A recent report (12) demonstrating the exclusive localization of carnitine transacetylase in the mitochondria of various animal tissues does not support the view that acetylcarnitine acts as the carrier of acetyl units across the mitochondrial membrane.

TABLE I

CONVERSION OF THE ^{3}H AND ^{14}C OF (1-^{3}H,1-^{14}C)-GLUCOSE, (6-^{3}H,6-^{14}C)-GLUCOSE, L-(3-^{3}H,3-^{14}C)-LACTATE AND (2-^{3}H,2-^{14}C)-ACETATE TO FATTY ACIDS BY LACTATING RAT MAMMARY GLAND SLICES

250 mg of lactating rat mammary gland slices were incubated for 3 hours at 37° in 2.5 ml of Krebs-Henseleit bicarbonate buffer (pH 7.3) containing 25 µmoles of labeled and/or unlabeled substrate as indicated below. The gas phase was 95% O_2 and 5% CO_2. The fatty acids (18) and lactic acid (19) were isolated and assayed for ^{3}H and ^{14}C activity in a Packard liquid scintillation spectrometer by the discriminator-ratio method of Okita et al. (26). Fatty acids were converted to sodium salts and subjected to Kuhn-Roth oxidation. The acetate representing the omega and the omega-minus-one carbon atoms of the fatty acids was isolated and assayed for ^{3}H and ^{14}C activity. Each value is the average and its standard error of results from 4 separate experiments with different rats.

Substrate		Per cent of utilized isotope recovered as:						
Labeled	Unlabeled	Fatty acids		Lactic acid		Terminal methyl group of fatty acids		
		^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	$^{3}H/^{14}C$
(1-^{3}H,^{14}C)-glucose	None	24.7±2.4	20.4±1.8	6.3±0.7	6.5±0.7	4.0±0.5	2.1±0.5	0.54±0.04
(6-^{3}H,^{14}C)-glucose	None	48.7±5.0	12.4±1.3	8.5±1.0	7.4±0.7	9.5±1.6	5.0±0.9	0.54±0.03
L-(3-^{3}H,^{14}C)-lactate*	Glucose	74.5±4.2	17.5±0.9			12.2±2.6	7.3±1.3	0.61±0.03
(2-^{3}H,^{14}C)-acetate	Glucose	67.4±1.8	23.1±0.6	0	0	9.1±1.1	8.2±1.1	0.90±0.02

* L-(3-^{3}H)-lactate was synthesized by fermentation of (6-^{3}H)-glucose with L. casei (19); L-(3-^{14}C)-lactate was prepared by reduction (27) of purified (3-^{14}C)-pyruvate (28) with crystalline lactic dehydrogenase and DPNH. The isotopically labeled L-lactates were isolated by paper chromatography in radiochemically pure form.

from (1-^{3}H,1-^{14}C)-glucose, (6-^{3}H,6-^{14}C)-glucose, L-(3-^{3}H,3-^{14}C)-lactate and (2-^{3}H,2-^{14}C)-acetate (Table I). This tissue was used because of its exceptionally high capacity for lipogenesis. Lactating rats of the Long-Evans strain, 15-16 days postpartum having suckled at least 6 pups, were killed, and their inguinal mammary glands quickly excised. Washed gland slices were incubated in a Krebs-Henseleit bicarbonate buffer containing the isotopically labeled and unlabeled substrates, as shown in Table I. The manner of incubation (15), the fatty acid (18) and lactic acid (19) isolation procedures, and the isotopic assays (18) have been described. The fatty acids were oxidized by a modification (20) of the Kuhn-Roth procedure (21) that yields acetate from only the terminal methyl end. We found, as did D'Adamo (22), that labilization of the hydrogen from the terminal methyl group of the fatty acid occurs to the extent of 30% during this oxidation. Since the extent of labilization was constant under the conditions we used, all values reported in the last column of Table I were corrected for this exchange.

We realized that no valid assessment of the pathway of pyruvate conversion to fatty acids could be arrived at solely from a study of the values for the ratio of $\frac{^{3}\text{H in total fatty acids}}{^{14}\text{C in total fatty acids}}$ (Table I), since this ratio is largely influenced by (i) the loss of tritium from the internal and carboxyl carbons during the incorporation of malonyl-CoA into fatty acids and (ii) the considerable incorporation into fatty acids of tritium from TPN^{3}H formed from the one position of glucose via the pentose phosphate pathway (23). Thus, any loss of tritium from the terminal methyl group of the

fatty acids would be masked. The use of the ratio $\frac{^3H \text{ in terminal methyl of fatty acids}}{^{14}C \text{ in terminal methyl of fatty acids}}$ shown in the last column of Table I does not suffer from these limitations.

The values for the ratio $\frac{^3H \text{ in terminal methyl of fatty acids}}{^{14}C \text{ in terminal methyl of fatty acids}}$ (Table I) observed in the experiments with (2-^{3}H,2-^{14}C)-acetate were close to unity, whereas those observed in the experiments with (1-^{3}H,1-^{14}C)-glucose, (6-^{3}H,6-^{14}C)-glucose and L-(3-^{3}H,3-^{14}C)-lactate were between 0.5 and 0.6. This indicates that while little tritium from the methyl group of acetate exchanged with hydrogen when acetate was converted to acetyl-CoA, considerable exchange did occur at this position of the acetyl-CoA derived from glucose or lactate. Tritium from the 1 or 6 position of glucose may be lost at the phosphoenolpyruvate stage before this hexose is converted to pyruvate (24), but the similarities in the values for the ratio $\frac{^3H \text{ in terminal methyl of fatty acids}}{^{14}C \text{ in terminal methyl of fatty acids}}$ in the experiments with (1-^{3}H,1-^{14}C)-glucose, (6-^{3}H,6-^{14}C)-glucose and L-(3-^{3}H,3-^{14}C)-lactate suggest that such hydrogen loss in lactating rat mammary gland is of minor importance. A similar conclusion was reached previously (23). The tritium loss we observed in the terminal methyl group of the fatty acids derived from labeled glucose and labeled lactate might also be due to an exchange of hydrogen bound to the methyl carbon of pyruvate when pyruvate is converted to alanine (25). Reversibility of this transamination reaction would result in loss of tritium on the methyl carbon of pyruvate before conversion of pyruvate to acetyl-CoA. However, the calculated values for the ratio

$\frac{^3H \text{ in lactate}}{^{14}C \text{ in lactate}}$ in the experiments with (1-^{3}H,1-^{14}C)-glucose and with (6-^{3}H,6-^{14}C)-glucose were close to unity (columns 5 and 6, Table I). These latter findings indicate that reversible transamination could not account for a value of less than one for the ratio $\frac{^3H \text{ in terminal methyl of fatty acids}}{^{14}C \text{ in terminal methyl of fatty acids}}$ derived from the labeled glucose and labeled lactate (last column, Table I).

Thus, our observations on the conversion of glucose and lactate to fatty acids by the lactating mammary gland are consistent only with the last of the four possibilities presented, i.e., scheme (d), in which the loss of hydrogen that occurs when carbons 1 or 6 of glucose and carbon 3 of lactate are converted to the terminal methyl group of fatty acids is the result, most likely, of citrate formation.

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