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*CITRATE CLEAVAGE IN ADIPOSE TISSUE**

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The synthesis of fatty acids from acetyl CoA is an extramitochondrial process[1, 2] but the production of acetyl CoA from pyruvate occurs within the mitochondria. This means that if lipogenesis from glucose is to proceed within the cell, a transfer of acetyl CoA across the mitochondrial membrane must occur. It has been proposed that this transfer does not occur directly but by the condensation of acetyl CoA with oxaloacetate to form citrate. The citrate then diffuses across the mitochondrial membrane and in the extramitochondrial space undergoes the following reaction catalyzed by the citrate cleavage enzyme.[3]

$$\text{Citrate} + \text{CoA} + \text{ATP} \rightarrow \text{Acetyl CoA} + \text{Oxaloacetate} + \text{ADP} + \text{Pi}.$$

The operation of this pathway in liver is supported by evidence that both the rate of citrate incorporation into fatty acids and the activity of the citrate cleavage enzyme vary with nutritional and hormonal states in which the role of lipogenesis is altered.[2, 4, 5] Since adipose tissue is capable of active lipogenesis, it seemed of importance to investigate the amount of citrate cleavage enzyme, its variation with diet, and its possible role in rat epididymal (white) and interscapular (brown) adipose tissue. The results obtained support a function for this enzyme in adipose tissue similar to that suggested for liver.

Materials and Methods.—All rats used were males of the Sprague-Dawley strain, supplied by Holtzman Co. (Madison, Wisconsin) and maintained as de-

scribed previously.[6] At the time of sacrifice by decapitation, all animals weighed 130–255 gm. Purina laboratory chow for mice, rats, and hamsters constituted the basic diet. Where high-carbohydrate feeding is specified, pelleted "fat-free" test diet of Wooley and Sebrell,[7] supplied by Nutritional Biochemicals Corp., was employed. It contained 58 per cent sucrose, 21 per cent casein, 16 per cent cellulose, and a mixture of salts and vitamins.

Homogenates of white (epididymal) and brown (interscapular) adipose tissue were prepared at room temperature in 0.25 *M* sucrose with a Ten-Broeck homogenizer. Homogenates of white adipose tissue were 20 per cent (w/v), of brown, 5–10 per cent. Aliquots of unfractionated homogenates were reserved for nitrogen analysis by a micro-Kjeldahl method.[8] The homogenates were then centrifuged in a Servall refrigerated centrifuge at 40,000 × *g* for 10 min at 0–3°. The clear supernatant fluid was withdrawn with a capillary dropper.

Citrate cleavage activity of the supernatant was assayed at 37° by a modification of the malic dehydrogenase method of Srere,[9] using a Beckmann DU spectrophotometer. Each cuvette contained in a total volume of 3.0 ml: 100 μmoles tris buffer, pH 7.3; 30 μmoles $MgCl_2$; 30 μmoles mercaptoethanol; 3 μmoles KCN; 30 μmoles K citrate; 0.5 mg (approximately 0.6 μmole) coenzyme A; 10 μg malic dehydrogenase; approximately 0.25 μmole NADH; tissue extract; and 15 μmoles ATP, which was added last. Absorbancy changes at 340 mμ were measured against a blank containing all reagents but NADH and ATP. Results are expressed as μmoles citrate cleaved/mg whole homogenate nitrogen/hr. Coenzyme A, ATP, NADH, and malic dehydrogenase were obtained from Sigma Chemical Co. All other chemicals were of reagent grade.

Results.—Citrate cleavage enzyme activity in epididymal adipose tissue from normal rats fed a chow diet has an average value of 14.9 μmoles/mg homogenate nitrogen/hr. This value is not significantly diminished by a 3-day fast (Fig. 1). If animals which have been starved for 3 days are then refed with a diet high in carbohydrate and low in fat, a 5.8-fold increase in activity is observed, with a maximum between 6 and 10 days of refeeding and a smaller peak at 3 days (Fig. 1). Values for citrate cleavage activity of brown adipose tissue are also shown in Figure 1. These are somewhat lower than the corresponding values for white adipose tissue, but adhere to the same pattern of increase with refeeding.

If the high-carbohydrate diet is administered to animals which have previously been maintained on the chow diet, without an intervening fast, citrate cleavage enzyme activity of white adipose tissue increases over a period of 3 days to 54.0 μmoles/mg homogenate nitrogen/hr (Fig. 2). This value, which represents a 3.6-fold increase over that of chow-fed animals, persists for the subsequent 7 days of the experimental period.

The pattern of nutritional variation of citrate cleavage enzyme of adipose tissue closely resembles that of the liver enzyme, with the exception that a 3-day fast which reduces the hepatic value by half has no effect on the adipose tissue value. The per cent increase observed with high-carbohydrate refeeding is greater for the liver enzyme, although citrate cleavage per mg homogenate nitrogen is higher in adipose tissue. The highest value for hepatic citrate cleavage enzyme reported by Kornacker and Lowenstein[4] was 6 μmoles/mg high-speed supernatant protein/hr and occurred after 2 days of starvation and 3 days of refeeding a high-carbohydrate

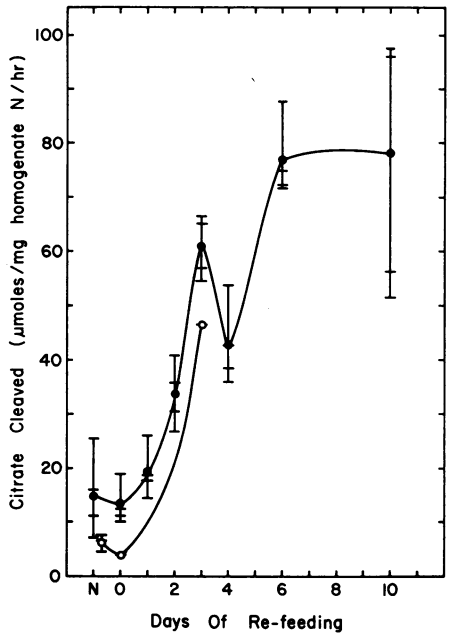

FIG. 1.—Citrate cleavage activity in rat adipose tissue: effect of refeeding a high carbohydrate–low fat diet to animals starved for 3 days. Each determination is represented by a short horizontal line. The average value for each set is represented by a circle—black circles, epididymal (white) adipose tissue; white circles, interscapular (brown) adipose tissue. Normal values (*N*) are shown for comparison.

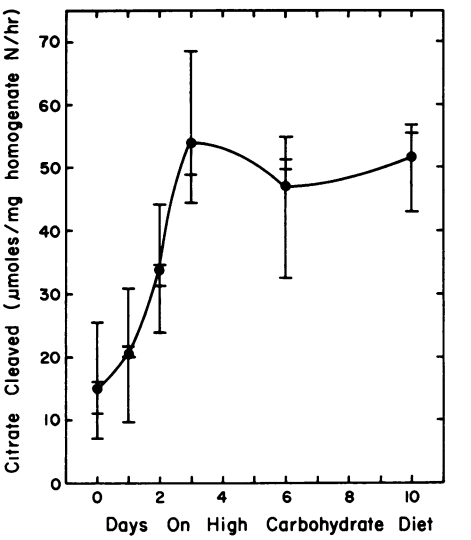

FIG. 2.—Citrate cleavage activity in epididymal adipose tissue of rats transferred from chow diet to high carbohydrate–low fat diet. Each determination is represented by a short horizontal line. The average value for each set is represented by a circle.

diet. In order to convert this value to the terms employed here, we have determined that 1 mg of high-speed liver supernatant protein equals 3.3 mg of total homogenate nitrogen. Thus a value of 20 μmoles/mg homogenate nitrogen/hr is obtained for rat liver, which may be compared with a value of 60 in white adipose tissue from rats starved for 3 days and refed for 3 days on a similar diet.

Discussion.—The activity of the citrate cleavage enzyme in adipose tissue exceeds that of any other tissue so far studied in the rat when comparisons are made on a tissue nitrogen basis. In addition, nutritional conditions which are known to stimulate lipogenesis from carbohydrate are found to increase the activity of this enzyme in adipose tissue. There is thus a marked resemblance of the citrate cleavage enzyme and the malic enzyme[10] in regard to their high activity in adipose tissue and response to dietary conditions. Indeed, the ratio of the activity of the two enzymes in adipose tissue varies only slightly during the substantial increases in activity which occur with high carbohydrate–low fat refeeding (Table 1).

These findings strongly suggest that citrate cleavage enzyme and malic enzyme operate together in lipogenesis. This suggestion is supported by data which show that rat liver, a tissue involved in lipogenesis, is rich in both of these enzymes, and that their activity in liver also undergoes parallel and striking changes with dietary alterations which affect lipogenesis.[4, 5, 10–13] In mammary gland the transition

TABLE 1

COMPARISON OF CITRATE CLEAVAGE ENZYME AND MALIC ENZYME IN RAT EPIDIDYMAL ADIPOSE TISSUE

Nutritional condition	Malic enzyme	Citrate cleavage enzyme	Ratio malic enzyme to citrate cleavage enzyme
Normal chow-fed	38.9 (4)	14.9 (5)	2.61
Fasted 3 days	20.8 (4)	13.2 (4)	1.58
Fasted 3 days, refed high carbohydrate diet 3 days	129.3 (5)	60.7 (4)	2.13
Fed high carbohydrate diet 3 days	121.0 (3)	54.0 (3)	2.24

Values for malic enzyme activity are taken from Wise and Ball (1964).[10] All activities are expressed as μmoles product formed/mg whole homogenate nitrogen/hr. The number of experiments is given in parentheses.

from prepartum conditions to active lactation is reflected in large increases of both enzymes. Malic enzyme levels are elevated 8-fold in lactating mammary gland,[14] and citrate cleavage enzyme levels are increased 12-fold.[15]

A possible role for the citrate cleavage enzyme and the malic enzyme in lipogenesis is illustrated by the diagram shown in Figure 3. Here the conversion of glucose to pyruvate is pictured as occurring in the extramitochondrial space. Pyruvate enters the mitochondria where it may either be oxidized to acetyl CoA or converted to oxaloacetate by pyruvate carboxylase, which is known to be present in both liver[16] and adipose tissue.[10] The acetyl CoA and oxaloacetate produced condense to form citrate which can either be oxidized to CO_2 or diffuse out of the mitochondria. In the latter case it encounters the citrate cleavage enzyme and is reconverted to acetyl CoA and oxaloacetate. The oxaloacetate may then be reduced to malate by the action of malate dehydrogenase. NADH for this process can be supplied by the extramitochondrial reactions in which glucose is converted to pyruvate. The malate formed reacts with NADP and the malic enzyme to produce pyruvate, CO_2, and NADPH. This reduced coenzyme may be utilized for reduction of acetyl CoA to fatty acids. The cycle is

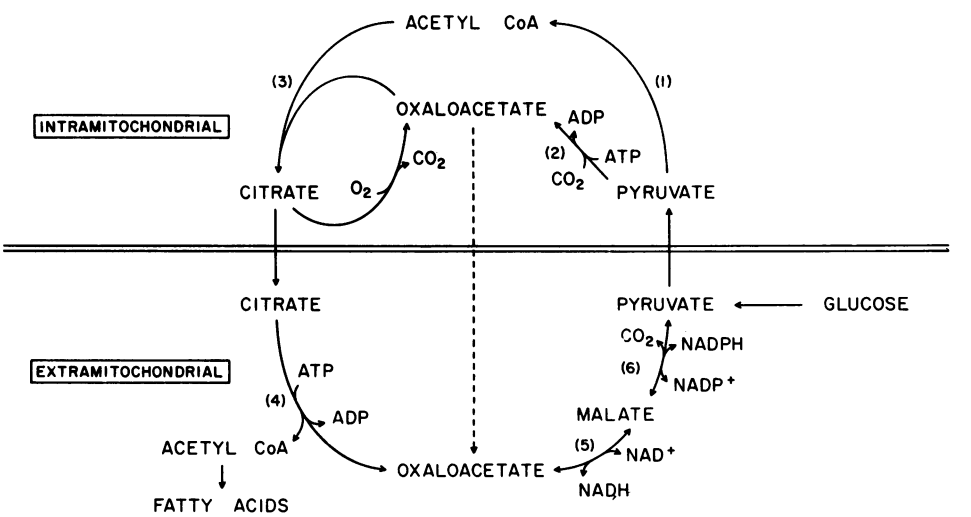

FIG. 3.—Postulated scheme for lipogenesis from glucose in adipose tissue. The reactions indicated are catalyzed by (1) pyruvate oxidase; (2) pyruvate carboxylase; (3) citrate condensing enzyme; (4) citrate cleavage enzyme; (5) malate dehydrogenase; and (6) malic enzyme.

then complete with the net result that one pyruvate molecule has been converted to acetyl CoA, and one molecule of NADH has been converted to NADPH.

Each turn of this cycle requires that two high-energy phosphates be supplied, one for pyruvate carboxylation and the other for citrate cleavage. Theoretically these requirements could be met by the two high-energy phosphates that should result from oxidation by O_2 of the reduced flavoprotein produced during the conversion of pyruvate to acetyl CoA. The cycle should therefore be energetically self-sufficient. It will be limited in its operation, however, because the cycle has a maximum yield of one molecule of NADPH per molecule of acetyl CoA generated. This is approximately half the quantity of NADPH needed to convert acetyl CoA to fatty acids. The remainder of the NADPH required for lipogenesis could conceivably be produced either by a foreshortened cycle in which oxaloacetate diffuses from the mitochondria (represented by the broken line in Fig. 3) or by the pentose cycle.

Previous studies from our laboratory are in agreement with the operation of a citrate-malate cycle in adipose tissue. Flatt and Ball[17] found that 37 per cent of the reduced coenzymes used during insulin-stimulated lipogenesis were supplied by a source other than the pentose cycle. In the presence of both insulin and epinephrine this value rose to 47 per cent or nearly the theoretical maximum to be obtained by the operation of the cycle discussed here. Also, as first shown by Jungas and Ball,[18] there is a marked increase in oxygen consumption during lipogenesis stimulated by insulin in adipose tissue. In the experiments of Flatt and Ball[17] the increase in oxygen consumption during insulin-stimulated lipogenesis amounted to 1.65 μmoles O_2 (Table 3), while the increase in incorporation of acetyl CoA into fatty acids was 3.77 μmoles (Fig. 3). This corresponds to 0.437 μmoles of O_2 per μmole of acetyl CoA reduced to fatty acid. If it is assumed that this increase in oxygen consumption represents the formation of two $\sim P$ connected with pyruvate oxidation, it would account for the production of 0.874 μmoles of NADPH by the transhydrogenation cycle outlined here. This would represent 52 per cent of the 1.674 μmoles needed for the reduction of 1 μmole of acetyl CoA as calculated by Flatt and Ball.[17] Thus the operation of the proposed cycle in adipose tissue would be in good quantitative agreement with the observations of Flatt and Ball.[17]

The use of NADPH rather than NADH for fatty acid synthesis imposes upon the cell a requirement for reoxidation of the reduced coenzymes formed in the production of acetyl CoA from glucose. The extramitochondrial nature of lipogenesis introduces a demand for acetyl group transport across the mitochondrial membrane. Oxidation-reduction balance, with simultaneous transfer of the acetyl group from its site of formation to its site of utilization, could be achieved by the proposed citrate-malate cycle.

Summary.—The activity of the citrate cleavage enzyme expressed in terms of tissue nitrogen content is higher in rat adipose tissue than in any other tissue examined. This activity may be markedly increased by fasting and refeeding the animals a high carbohydrate–low fat diet or changing them to such a diet from a commercial chow diet. These results are consistent with a proposed role for this enzyme in lipogenesis. Considered in conjunction with previous data, they permit the construction of an integrated chain of reactions describing the process of lipogenesis from glucose at it occurs in rat adipose tissue.

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*GENETIC IMPLICATIONS OF PERIODIC PULSATIONS OF THE RATE OF SYNTHESIS AND THE COMPOSITION OF RAPIDLY LABELED BACTERIAL RNA**

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A recent paper from this laboratory[1] described the observation of rhythmic alternations in the rate of synthesis and the composition of rapidly labeled RNA formed in consecutive segments of the generation period of the synchronously growing donor strain HfrH of *Escherichia coli* K12. When a short pulse of P^{32}-orthophosphate was given at regular intervals during a growth cycle, two spurts of the rates of synthesis occurred, coincident with the periods when approximately 40 and 80 per cent of the DNA had been duplicated; and the RNA newly produced during these periods resembled ribosomal RNA in its apparent nucleotide composition. The RNA specimens formed during other segments of the growth cycle, though exhibiting slight, but characteristic, periodic variations, were, on the whole, similar to the DNA of *E. coli* with regard to nucleotide proportions and sometimes also to base pairing. These findings suggested to us that the general process of the transcription of genetic information is ordered and attuned to the consecutive phases of chromosomal duplication,[2-4] and that the periodic synthesis of ribosomal RNA may indicate the periods at which ribosomal genes become available for transcription.

When our observations were projected on the known linkage map of strain HfrH,[5] one of the RNA peaks appeared to be near the streptomycin locus. It appeared, therefore, of interest to ascertain whether another Hfr strain having a