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THE INCORPORATION *IN VITRO* OF [^{14}C]PALMITATE INTO GLYCEROLIPIDS BY VARIOUS CELL COMPONENTS

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SUMMARY

1. The incorporation *in vitro* of [^{14}C]palmitate into triglycerides, ethanolamine phosphatides, and choline phosphatides has been studied. Whole homogenates, mitochondria, and microsomes plus cytoplasmic supernatant from normal and 3-day "regenerating" rat livers were employed as enzyme.

2. The relative uptakes of ^{14}C in the various lipids differed, depending on the part of the cell used as enzyme, and whether or not regenerating liver or normal liver was employed.

3. The data suggest a multiplicity of pathways of incorporation of fatty acid into the glycerolipids, and indicate changes in lipid metabolism in response to altered tissue physiology.

INTRODUCTION

The results of earlier studies[1,2] on the incorporation *in vivo* of [^{14}C]acetate and [^{14}C]glycerol into phospholipids and triglycerides of tissues undergoing cell division were interpreted as indicating either that different pools of fatty acids existed which possessed different availabilities for the formation of the various glycerolipids, or that there were pathways of formation other than those that had been described[3-6] as involving a diglyceride precursor common to phospholipids and triglycerides. Were the latter the case, results of the earlier experiments suggested that the nature of the net synthetic process depended in part, at least, on the physiological states or relative amounts of the different sub-cellular components present in a tissue.

In an effort to understand better the relative involvements of the different components of the cell in the metabolism of some of the glycerolipids, a study has been made of the incorporation of [^{14}C]palmitate *in vitro* into triglycerides, and choline and ethanolamine phosphatides by whole homogenate, a microsomal preparation, and mitochondria of normal and "regenerating" rat liver. The latter was studied in view of the differences observed earlier in lipid metabolism by this tissue[1,2,7,8].

EXPERIMENTAL

3–6-month-old male Holtzman rats that had been kept on a commercial stock ration were employed. Regenerating livers were harvested 3 days following partial hepat-

ectomy. At that time large numbers of mitotic figures are present[7], and there is a greatly increased uptake *in vivo* of [^{32}P]phosphate (see refs. 7 and 8), [^{14}C]acetate[1,2], and [^{14}C]glycerol[2], in the tissue lipids. Livers were perfused under anesthesia with cold 0.9 % NaCl prior to sacrifice of the animals.

Mitochondria and microsomal supernatant (that which remained after the sedimentation of mitochondria) were prepared by the method of TIETZ AND SHAPIRO[9]. Whole liver homogenates were prepared in 0.25 M sucrose buffered at pH 7.4 with Tris. The equivalent of 0.5 g (homogenate), 4 g (mitochondria), or 0.67 g (microsomal supernatant) of tissue were employed in each flask, in a final volume of 4.8 ml. The incubation mixture contained 50 μmoles of glycerol, 20 μmoles of magnesium chloride, 100 μmoles of sodium succinate, 50 μmoles of Tris buffer (pH 7.4), 20 μmoles of phosphate buffer (pH 7.4), 10 μmoles of ATP, and 2 μmoles of [1-^{14}C]palmitic acid (390000 counts/min) in a final volume of 4.8 ml. The incubations were carried out at 37° in 50-ml erlenmeyer flasks, with shaking in air. Zero-time controls were used in which the reaction was stopped by the addition of 20 ml of ethanol immediately after adding the tissue preparation to the mixture.

At the end of the incubation periods, 20 ml of methanol were added to each flask and the contents heated at 55–60° for 10 min. 10 ml of $CHCl_3$ were added and the heating continued for another 10 min. The flasks were cooled, the contents centrifuged, and the residues extracted three times with ethanol–ether (3:1). The extracts were combined, evaporated *in vacuo*, and the lipids finally taken up in light petroleum (boiling range, 30–60°). The solutions were dried over anhydrous Na_2SO_4, and the lipids dissolved in 0.5 ml $CHCl_3$ which contained 0.5 mg palmitic acid. The phospholipids were precipitated three times in the cold with acetone, and finally dissolved in light petroleum, and phosphatidyl ethanolamine and phosphatidyl choline separated by chromatography on silicic acid[2].

The acetone-soluble portions were reduced to a volume of 3–4 ml under nitrogen, and 20 mg palmitic acid dissolved in 1 ml ethanol was added, followed by 1 ml hot ethanol containing 20 mg lead acetate. The tubes were chilled in ice for 1 h and centrifuged, and the precipitates washed once with acetone and the extract and washings combined. This was repeated 3 times, and provided a satisfactory removal of the radioactive free fatty acid. The triglycerides were prepared by chromatography on silicic acid[10]. Plating of samples, counting procedures, and P determinations have been described elsewhere[1,11].

RESULTS

Incubation periods of 5 min to 1 h were employed. The significant results for the purpose of this study may be obtained from the shorter time periods, and are shown in Figs. 1–3. The data have been calculated to show incorporation of radioactivity by tissue preparations equivalent to 1 g of original liver.

The relative magnitudes of uptake of radioactivity into the lipids, using whole normal liver homogenate or mitochondria were ethanolamine phosphatide > choline phosphatide > triglyceride. With mitochondria it was ethanolamine phosphatide = choline phosphatide > triglyceride, and when microsomal supernate was employed, the order was choline phosphatide > ethanolamine phosphatide > triglyceride.

When the enzyme preparations were obtained from regenerating livers the amounts of radioactivity incorporated into the lipids by each of the three tissue

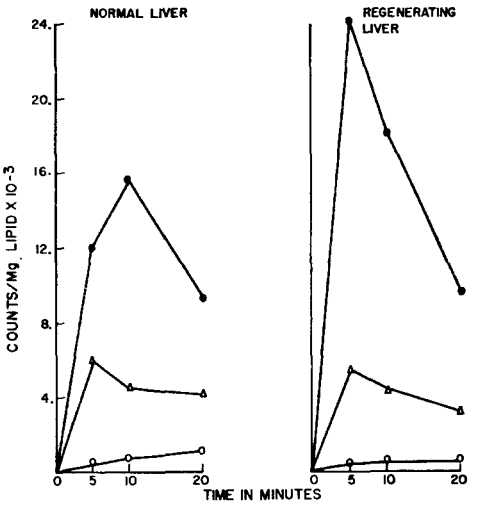

Fig. 1.

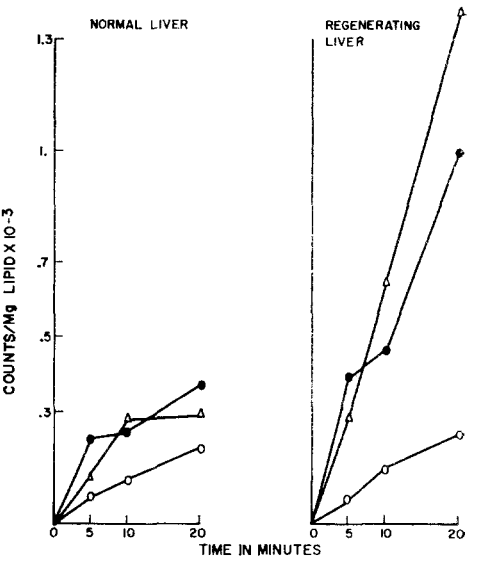

Fig. 2.

Fig. 1. Incorporation *in vitro* of [^{14}C]palmitate into lipids, using whole liver homogenate as enzyme. The explanation of the curve is as follows: O—O, triglycerides; Δ—Δ, choline phosphatides; ●—●, ethanolamine phosphatides. The data have been calculated to show the incorporation by homogenate equivalent to 1 g original tissue.

Fig. 2. Incorporation *in vitro* of [^{14}C]palmitate into lipids, using liver mitochondria as enzyme. See Fig. 1 for explanation of curves. The data have been calculated to show the incorporation by the mitochondrial equivalent of 1 g original tissue.

Fig. 3. Incorporation *in vitro* of [^{14}C]palmitate into lipids, using liver microsomal supernatant (see text) as enzyme. See Fig. 1 for explanation of curves. The data have been calculated to show the incorporation by the microsomal supernatant of 1 g original tissue.

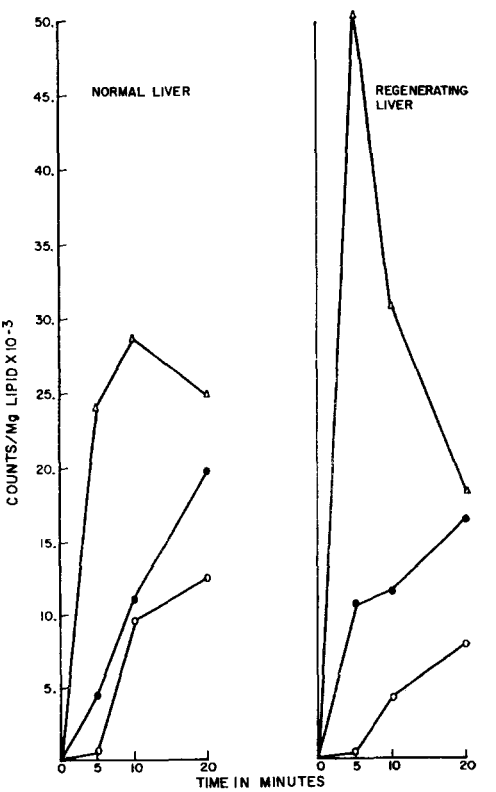

Fig. 3.

preparations bore the same relationships to one another as those described for normal liver preparations. A number of differences otherwise was observed, however. For example, when whole regenerating liver homogenate was employed the amounts of incorporation of ^{14}C into the choline phosphatides and triglycerides were the same as those observed with normal liver homogenate, but the uptake in the ethanolamine phosphatides was greatly increased (Fig. 1). Employing mitochondria from regenerating liver, there were increased uptakes in both ethanolamine and choline phosphatides, over that seen with normal liver, and no change in the triglycerides (Fig. 2). With regenerating liver microsomal supernatant as enzyme there was a marked increased incorporation of [^{14}C]palmitate into the choline phosphatides and a decrease in the triglycerides (Fig. 3). Thus, in regenerating liver tissue, at a time when considerable mitotic activity is present, the mitochondria exhibit an increased capacity to incorporate [^{14}C]palmitate into both of the phosphatides; the microsomal supernatant increases radioactivity uptake in choline phosphatides while decreasing it in ethanolamine phosphatides and triglycerides, and the whole regenerating liver homogenate increases the incorporation of ^{14}C into the ethanolamine phosphatides over that observed with normal tissue, with no change occurring in choline phosphatides or triglycerides.

The incorporation of radioactivity into the choline phosphatides and triglycerides by the microsomal supernatant exceeded greatly that by equivalent amounts of either whole homogenate or mitochondria. This was observed with both normal and regenerating liver. On the other hand, the whole homogenate incorporated more radioactivity into the ethanolamine phosphatides than did either of the other preparations. It would appear from these observations that there is a mechanism in whole tissue that actually suppresses the incorporation of [^{14}C]palmitate into choline phosphatides and triglycerides while exerting no effect on the ethanolamine phosphatides.

DISCUSSION

The data of Figs. 1–3 demonstrate clearly that the incorporation *in vitro* of [^{14}C]-palmitate into triglycerides, choline phosphatide, and ethanolamine phosphatide is accomplished to different degrees by liver mitochondria, the microsomal supernatant, and whole liver homogenate. Furthermore, altered physiological activity, occasioned in part, at least, by the increased mitotic activity in the regenerating liver[1,2] is accompanied by uptake patterns in the lipids examined which differ qualitatively from those observed with normal tissue preparations.

Some of the differences no doubt may be explained by the fact that the same incubating medium was used throughout, which was presumably more adequate for some of the preparations than for others. In this connection, it should be pointed out that neither coenzyme A nor α-glycerophosphate were employed. Experiments are under way to study the effect of these and other substances on the incorporation of [^{14}C]palmitate *in vitro* into glycerolipids by different cell components.

If one were to assume that the incorporation of palmitate into the lipids studied proceeded entirely via fatty acid–coenzyme A esterification to form a phosphatidic acid, thence to D-α,β-diglyceride[2,6], these data might be explained on the basis of differences in suppression or stimulation by the tissue preparations employed, of one of the final reactions leading from the diglyceride to one or another of the reaction

products. While this is a possibility, other pathways in glycerolipid formation have been described[12–16], and undoubtedly are of significance in the processes measured in the present experiments.

The results obtained with the regenerating liver preparations indicate some of the ways in which the organism responds biochemically to tissue regeneration. Another interesting example of a selective response, by way of altered lipid metabolism, is that observed when rats are fasted 18–24 h. Liver mitochondria from such animals lose much of their capacity to incorporate [^{14}C]palmitate *in vitro* into ethanolamine phosphatide, without any appreciable difference being seen in its incorporation into either choline phosphatide or triglycerides[17].

The extent to which data obtained from experiments performed *in vitro* can be extrapolated to the situation obtaining *in vivo* is not known. It seems reasonable to assume, however, that each entity within a cell adds its metabolic activities to or exerts a control over those of the others, and the differing catabolic emphases noted in the different enzyme preparations employed in the present experiments suggest a possible mode of control over the incorporation of fatty acids into the lipids examined. For example, a reduction in the mitochondrial population such as that found in tumors[18] conceivably might result in relatively less incorporation of fatty acid into ethanolamine phosphatide and relatively more into choline phosphatide.

The significance of such alterations in the rates of formation of these compounds in the economy of the cell or tissue is unknown, and is the ultimate goal of these as well as the investigations of others.

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