FORMATION OF GLYCERIDES WITH SATURATED AND UNSATURATED FATTY ACIDS IN RAT-LIVER PARTICLES

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(Received November 27th, 1958)

SUMMARY

The incorporation into neutral glycerides of [1-^{14}C]palmitic acid by mitochondrial and by microsomal enzyme systems of rat liver exceeded markedly that of stearic and linoleic acids.

When the enzyme systems were supplied with mixtures of palmitic and oleic or with palmitic and linoleic acid, the incorporation of palmitic acid was depressed much beyond the dilution factor and the unsaturated fatty acids were preferred for triglyceride synthesis.

The lower incorporation of linoleic acid into neutral glycerides, when supplied as the only acid, was due to the accumulation of its incorporation products in the "phospholipid fraction", eluted by methanol from silicic acid columns.

In the presence of linoleic acid carrier more [1-^{14}C]palmitic acid was found in the "phospholipid fraction" than with palmitate as sole acid.

The identity of the substances in the "phospholipid fraction" is discussed.

INTRODUCTION

The enzymic syntheses of triglycerides and phospholipids from fatty acids have several steps in common, proceeding via the coenzyme-A-activated acid to phosphatidic acid and diglycerides[1,2]. They differ only in the last step, leading from diglycerides to triglycerides or to phospholipids. It is of considerable interest to determine the factors which push the reaction chain in one or the other direction. The type of the fatty acids involved in the synthesis might be one of these factors, since it has been shown that liver phospholipids have a characteristic composition as regards their component fatty acids[3]. Thus only unsaturated fatty acids were found in the α-position and only saturated ones on the β ester position of liver lecithin. Feeding [1-^{14}C]stearic acid caused a higher relative incorporation into phospholipids than did labelled palmitic and myristic acids, which were incorporated predominantly into the triglyceride fraction[4]. With the unsaturated fatty acids only small amounts appeared in the phospholipid fraction[5].

In the present paper, the effects of various fatty acids on the formation of esters *in vitro* were tested in the two enzyme systems from rat liver, which catalyze the incorporation of fatty acids into triglycerides[2]. The interaction of several fatty acids on the enzyme system, when supplied concurrently, was also examined.

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MATERIALS AND METHODS

Rat-liver homogenates, mitochondria and supernatant were prepared as described previously[2]. Microsomes were obtained by centrifuging the supernatant which remained after sedimentation of the mitochondria, at 0° for 30 min at 100,000 *g* in a Spinco centrifuge.

Enzyme system A refers to the "mitochondrial system"[2]. It was composed of 0.5 ml mitochondrial suspension, 1.5 ml boiled supernatant, 0.1 ml native supernatant, 10 μmoles of K-ATP, 20 μmoles K-phosphate buffer pH 7.5, 10 μmoles $MgCl_2$ with the indicated fatty acids and made up to 3 ml with KCl-Tris-buffer[2].

Enzyme system B refers to the "microsomal system"[6]. It contained 0.25 ml of the supernatant, 10 μmoles sodium-α-glycerophosphate, 0.1 mg coenzyme A (Nutritional Biochemicals Corp.) and ATP, $MgCl_2$ and fatty acids as in system A.

Enzyme system C was similar to system B, but instead of the supernatant, lyophylized microsomes in the indicated amounts were used.

Incubation was carried out at 37° for 15 min.

Extraction and separation of triglycerides, phospholipids and fatty acids were carried out as before[2], unless otherwise described.

Radioactive fatty acids were obtained from the Radiochemical Centre, Amersham, England.

Carrier fatty acids were of commercial source, except for the linoleic acid, which was prepared according to PARKER *et al.*[7].

RESULTS

In the first set of experiments, it was examined how various carrier fatty acids will affect the incorporation of [1-^{14}C]palmitic acid into neutral glycerides. It is evident from Table I that the addition of palmitic acid carrier decreased the percentage incorporation of [1-^{14}C]palmitic acid proportionally to the increase in total concentration. Thus the total amount of palmitic acid incorporated was not changed significantly by changing its concentration in the reaction medium. This, however, was not the case when the palmitic acid carrier was replaced by oleic or linoleic acid.

TABLE I

INHIBITION OF PALMITIC ACID INCORPORATION INTO NEUTRAL GLYCERIDES BY OLEIC AND LINOLEIC ACIDS

For composition of reaction mixtures and conditions of incubation, see METHODS.

Enzyme system	[^{14}C]*fatty acid added 0.4 μmole*	*Carrier acid added 0.6 μmole*	*Incorporation of* [^{14}C]*acid*	
			% of counts	*10^{-3} μmoles*
A	palmitic	—	30	120
A	palmitic	palmitic	14	140
A	palmitic	oleic	5	20
A	palmitic	linoleic	5	20
B	palmitic	—	30	120
B	palmitic	palmitic	12	120
B	palmitic	oleic	5	20
B	palmitic	linoleic	3.5	14

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In spite of the fact that the total fatty acid concentration remained the same, a much lower percentage of [^{14}C]palmitic acid was incorporated into neutral glycerides and the total amount incorporated dropped accordingly. This was true for both enzyme systems tested, the mitochondrial (A) as well as the microsomal one (B).

An attempt was then made to ascertain whether this inhibition of palmitic acid esterification by the unsaturated fatty acids was the result of an overall inhibition of esterification, or whether it was confined to a decrease in the amount of palmitic acid esterified, the unsaturated acid displacing palmitic acid from the esterified compounds. In order to be able to measure the incorporation of palmitic acid and linoleic acid when present at the same time, experiments were set up in which [1-^{14}C]palmitic acid was incubated with linoleic acid carrier, while [1-^{14}C]linoleic acid was incubated with palmitic acid carrier, as demonstrated in Table II (Mixtures c and d).

TABLE II

INCORPORATION INTO NEUTRAL GLYCERIDES USING MIXTURES OF PALMITIC AND LINOLEIC ACID

For conditions and composition of reaction mixtures, see METHODS.

Expt. No.	*Enzyme system*	[^{14}C]*fatty acid added 0.4 μmole*	*Carrier acid added 0.4 μmole*	*Incorporation of [^{14}C]acid*		*Total incorporation 10^{-3} μmoles*
				% of counts	*10^{-3} μmoles*	
1	A	(a) palmitic	palmitic	14.5	116	116
	A	(b) linoleic	linoleic	8.0	64	64
	A	(c) palmitic	linoleic	5.2	20.8	97.6
	A	(d) linoleic	palmitic	19.2	76.8	
2	6 mg C	(a) palmitic	palmitic	23.0	184	184
	6 mg C	(b) linoleic	linoleic	12.0	96	96
	6 mg C	(c) palmitic	linoleic	8.0	32	132
	6 mg C	(d) linoleic	palmitic	25.0	100	
3	12 mg C	(a) palmitic	palmitic	35.0	280	280
	12 mg C	(b) linoleic	linoleic	20.0	160	160
	12 mg C	(c) palmitic	linoleic	29.0	116	220
	12 mg C	(d) linoleic	palmitic	26.0	104	

Since both mixtures were chemically identical, the sum of the [^{14}C]palmitic acid incorporation, found in mixture c, and that of [^{14}C]linoleic acid incorporation, obtained from mixture d, presents the overall formation of neutral glycerides in the mixture of these two acids. As is evident from Table II, in all cases this total incorporation was intermediary between that with palmitic acid alone (mixtures a) and linoleic acid alone (mixtures b), when present at the same total concentration. There was therefore no overall inhibition of the esterification process. However, it is also evident that out of the mixture of the two acids, linoleic acid is preferred and makes up the bulk of the acids esterified, while the incorporation of palmitic acid into triglycerides was largely depressed, as has already been shown in Table I. This displacement of palmitic acid by linoleic acid was found with both the mitochondrial and the microsomal system (Expts. 1 and 2) but became less and less evident as the enzyme concentration was increased (Expt. 3).

The preference of the enzyme systems for linoleic acid seemed at first difficult to reconcile with the additional finding that the incorporation of linoleic acid into

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triglycerides was generally lower than that of palmitic acid when both were the sole acids present (mixtures a and b). In spite of the fact that the affinity of linoleic acid for some component of the enzyme system exceeded that of palmitic acid it formed less of the final product. This discrepancy could be explained by assuming that linoleic acid, after going through the initial steps of the reaction chain leading to triglycerides, either accumulates as one of the intermediates of this chain or is diverted into alternative routes, for instance into phospholipids.

When the reaction products present in the ethanol–ether extracts of the various reaction mixtures were subjected to fractionation on a silicic acid column, according to BORGSTROEM[8], it was found (Table III), that the petroleum ether eluate, which contained the cholesterol esters, was almost inactive in all the mixtures tested. After elution of the triglycerides and unreacted fatty acids by chloroform, the rest of the activity could be eluted with methanol. This methanol extract, which contained the phospholipids, was markedly higher in activity in the mixtures containing linoleic acid than in those containing palmitic acid. The conversion of [1-^{14}C]palmitic acid into substances belonging to the "phospholipid fraction" was also enhanced by the presence of linoleic acid.

TABLE III

CHROMATOGRAPHIC FRACTIONATION OF THE REACTION PRODUCTS

The alcohol–ether extracts from expts. similar to those presented in Table II were evaporated and the lipids were dissolved in petroleum ether and chromatographed on a silicic acid column, according to BORGSTROEM[8]. The petroleum ether eluate contained the cholesterol esters, the chloroform eluate the neutral fat and fatty acids, and the methanol eluate is referred to as the "phospholipid fraction". The neutral glycerides were separated from unesterified fatty acid by passing their acetone solution through a magnesium oxide–celite column, as described in previous publications[2].

Expt. No.	*Enzyme system*	*[^{14}C]fatty acid added 0.4 μmole*	*Carrier acid added 0.4 μmole*	*% incorporation of [^{14}C]acid into*		
				cholesterol esters	*neutral glycerides*	*"phospholipid fraction"*
1	6 mg C	palmitic	palmitic	negligible	30	22
	6 mg C	palmitic	linoleic	negligible	20	30
2	12 mg C	palmitic	palmitic	negligible	33	33
	12 mg C	palmitic	linoleic	negligible	28	42
	12 mg C	linoleic	linoleic	negligible	12	62

TABLE IV

COMPARISON OF INCORPORATION INTO GLYCERIDES OF PALMITIC AND STEARIC ACIDS

For conditions and composition of reaction mixtures, see METHODS.

Enzyme system	*[^{14}C]fatty acid added 0.4 μmole*	*Incorporation of [^{14}C]acid*	
		% of counts	*10^{-3} μmole*
A	palmitic	26	104
A	stearic	12	48
B	palmitic	50	200
B	stearic	30	120

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In an additional set of experiments it was also found that stearic acid is much less effectively incorporated into triglycerides than palmitic acid (Table IV). This again was true for the mitochondrial and for the microsomal system.

DISCUSSION

The results obtained suggest that linoleic and oleic acid have a higher affinity than palmitic acid for some enzyme involved in the reaction chain leading to triglycerides. In mixtures of the saturated and unsaturated acids, the latter are preferentially incorporated into triglycerides. At the same time the amount of palmitic acid displaced from glyceride formation was found in the "phospholipid fraction". With linoleic acid alone incorporation was directed predominantly towards the "phospholipid fraction". The nature of the main substance in this fraction is still uncertain. Previous experience with similar reaction mixtures by KORNBERG AND PRICER[9] and by ourselves[6] has shown that the main component accumulating in the "phospholipid fraction" was phosphatidic acid. It seems likely that a similar substance is the one which is enriched in the linoleic acid experiments. This could not be proved conclusively by chromatography on silicic-acid-impregnated paper[10], since the substance behaved somewhat differently from reference phosphatidic acid, prepared from egg lecithin according to KATES[11]. However, it is quite likely that phosphatidic acids containing different acid components will show differences in chromatographic behaviour.

If phosphatidic acid is the main product of linoleic acid incorporation, the results could be explained by assuming that with the unsaturated fatty acids the initial reactions leading to phosphatidic acid are accelerated. However, the phosphatase which splits phosphatidic acid into diglyceride and inorganic phosphate seems to be inhibited by the unsaturated fatty acids in the substrate and, therefore, phosphatidic acid accumulates. This assumption could not yet be tested owing to the lack of saturated and unsaturated phosphatidic acids as substrates.

ACKNOWLEDGEMENTS

We wish to thank Mr. G. HOLENDER for his technical assistance.

This work was supported by a grant from the Hadassah Medical Organization, Jerusalem.

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