The synthesis of neutral glycerides by fractions of rat liver homogenates

In a previous communication[1] it has been shown that glyceride synthesis in liver is carried out by mitochondria upon enrichment with ATP, Mg^{++} and a factor in the supernatant.

Further examination of this factor revealed that the supernatant contains at least two components necessary for optimal activity: one is thermostable and present in the boiled supernatant, and the other is destroyed by heat. A small amount of the native supernatant is essential for the esterification of palmitic acid (Table I).

TABLE I

INCORPORATION OF 1-^{14}C-PALMITATE INTO NEUTRAL GLYCERIDES BY FRACTIONS OF RAT LIVER HOMOGENATES

Rat liver was homogenized with 3 vol. KCl-TRIS buffer (KCl, 0.154 M, 19 parts; tris(hydroxymethyl)aminomethane, 0.5 M, pH 7.5, 1 part; ethylenediaminetetraacetate, 10^{-4} M). Nuclei and cell debris were removed by centrifugation at low speed for 5 min and the homogenate was fractionated into particles (mitochondria) and supernatant ("native supernatant") by centrifugation at 10,000–12,000 r.p.m. in a Servall centrifuge. The particles were washed in buffer and resuspended in half the vol. of the original homogenate. Part of the supernatant was heated in a boiling water bath for 10 min and cleared by centrifugation ("boiled supernatant"). The reaction mixture consisted of 10 μmoles ATP; 20 μmoles potassium phosphate buffer, pH 7.5; 10 μmoles $MgCl_2$; 0.4 μmoles potassium 1-^{14}C-palmitate with the activity of 0.4 microcuries, with the indicated tissue fractions made up with KCl-TRIS buffer to 3 ml and incubated at 37° C with shaking for 60 min. Incorporation was measured as previously described[1].

Tissue fractions	*% ^{14}C-palmitate incorporated into neutral glyceride*
0.5 ml particles, 0.1 ml native supernatant and 1.5 ml boiled supernatant	33
Ditto without boiled supernatant	4.7
Ditto without native supernatant	6.5
Ditto without native supernatant and boiled supernatant	1.6

The boiled supernatant can be replaced by α-glycerophosphate and coenzyme A (Table II) (β-glycerophosphate and glycerol are inactive). In the presence of these two substances, native supernatant alone, in the absence of mitochondria, is sufficient to catalyze the incorporation of palmitate in neutral glycerides. On the other hand, the activity with mitochondria without added supernatant remains low. The active principle in the supernatant fraction can be precipitated by high speed centrifugation (40,000 r.p.m.) while none is retained in the supernatant thus obtained.

Palmitate incorporation into neutral glycerides may thus be brought about by two systems:

1. Mitochondria + native supernatant + boiled supernatant.
2. Native supernatant (or microsomes) + α-glycerophosphate + coenzyme A.

ATP and Mg^{++} are required in both systems.

One may conclude, therefore, that glyceride synthesis in liver is carried out by two independent mechanisms, one active in the mitochondria and the other in the microsomes. On the other hand, one may assume that the enzymes in both systems are identical, and that the entire enzymic activity, bringing about the incorporation of palmitate into neutral glycerides, is present in the microsome fraction. The mitochondria in system 1 would then act as a source for the glycerol moiety (either as glycerophosphate or another glyceride).

The assumption that α-glycerophosphate acts as glycerol donor in the microsomal synthesis of neutral glycerides is substantiated by the results presented in Table III, namely that the glycerol of 1-^{14}C-glycerophosphate is incorporated into neutral glycerides by the same enzyme system that incorporates palmitate. This is in agreement with the concept evolved by WEISS AND KENNEDY[2], that neutral glycerides are formed by a system similar to that giving rise to phospholipids, both proceeding via glycerophosphate and phosphatidic acid, by the following reaction steps:

1. Glycerophosphate + 2 Palmityl-SCoA $\longrightarrow$ Phosphatidic acid[3].
2. Phosphatidic acid $\longrightarrow$ Diglyceride + Phosphate.
3. Diglyceride + Palmityl-SCoA $\longrightarrow$ Triglyceride.
4. Diglyceride + Phosphoryl-choline $\longrightarrow$ Choline-phospho-lipid.

TABLE II

EFFECT OF α-GLYCEROPHOSPHATE AND COENZYME A ON PALMITATE INCORPORATION

(Conditions identical with those in Table I)

	Additions					% palmitate incorporated into neutral glycerides
	Particles (ml)	Native supernatant (ml)	Boiled supernatant (ml)	α-Glycerophosphate (ml of 0.05 M solution)	Coenzyme A (units)	
1.	0.5	0.1	1.5	—	—	31
	0.5	0.1	—	—	—	6
	0.5	0.1	—	0.2	30	42
	0.5	0.1	—	0.2	—	21
	0.5	0.1	—	—	30	8
	0.5	—	—	0.2	30	14
2.	0.5	0.1	1.5	—	—	17
	—	0.1	1.5	—	—	2.4
	0.5	0.1	—	0.1	30	51
	—	0.1	—	0.1	30	35
	—	0.5 ml "microsome fraction"*	—	0.1	30	36.5
	—	ditto	1.5	—	—	6.9
	0.5	ditto	—	0.1	30	43.5
	—	1.0 ml supernatant of microsomes	—	0.1	30	1.5

* Obtained by centrifugation of the "native supernatant" at 40,000 r.p.m. in a Spinco centrifuge.

TABLE III

INCORPORATION OF 1-^{14}C-GLYCEROPHOSPHATE IN NEUTRAL FAT FRACTION

Experiments were conducted under identical conditions as for radiopalmitate except that radioactive glycerophosphate was added with non-active palmitate. The alcohol-ether extract of the incubation mixture was washed with water, evaporated, the residue taken up in petroleum ether and precipitated with acetone in the presence of $MgCl_2$.

Additions				% 1-^{14}C-glycerophosphate incorporated into	
Particles (ml)	Native supernatant (ml)	1-^{14}C-Glycerophosphate (ml of 0.05 M solution)	Coenzyme A (units)	acetone-soluble fraction	acetone-insoluble fraction
0.5	0.1	0.1	30	6	1
0.5	0.1	0.1	0	0.5	0.15

According to this scheme, microsomal triglyceride synthesis differs from phospholipid synthesis only in the last reaction step (reaction 3 instead of 4). It remains to be shown whether the synthesis with mitochondria also proceeds by the same mechanism.

Y. STEIN
B. SHAPIRO

Department of Biochemistry, and Medical Department "B", Hebrew University, Hadassah Medical School, Jerusalem (Israel)

[1] A. TIETZ AND B. SHAPIRO, *Biochim. Biophys. Acta*, 19 (1956) 374.
[2] S. WEISS AND E. P. KENNEDY, *J. Am. Chem. Soc.*, 78 (1956) 3550.
[3] A. KORNBERG AND W. E. PRICER, Jr., *J. Biol. Chem.*, 204 (1953) 345.

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