dioxide, β-methylcrotonate and pyruvate. It was found that β-methylcrotonate and pyruvate were incorporated at a considerably lower rate than acetate, and that sucrose and carbon dioxide were not incorporated.

The dependency of acetate incorporation into rubber upon latex concentration in the incubation mixture is illustrated by the data of Table I. Latex was added to all tubes at the end of the incubation period and immediately inactivated by the addition of acetone so that equal amounts of rubber were present in all tubes for isolation and counting.

TABLE I

EFFECT OF ENZYME (LATEX) CONCENTRATION OF ACETATE INCORPORATION INTO RUBBER[a]

Latex, ml.	Radioactivity, (counts/minute/mg. carbon)
0	2.6
0.02	2.8
.04	4.5
.06	14
.08	38
.10	120
.13	210

[a] Reaction conditions as for Fig. 1 except for latex concentration as indicated; incubation time, 4 hours.

That the radiocarbon to acetate was in fact incorporated into rubber was demonstrated by degradation of a sample of the enzymatically synthesized rubber to levulinic acid. The specific activities of rubber bromide isolated as in the above experiments, of rubber bromide isolated after preliminary alcohol purification of the rubber, and of levulinic acid 2,4-dinitrophenylhydrazone prepared from the purified rubber were 220, 200, and 230 counts per minute per milligram of rubber carbon.

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THE ENZYMATIC SYNTHESIS OF TRIGLYCERIDES

Sirs:

Previous reports from this Laboratory have described the function of cytidine coenzymes in the biosynthesis of lecithin and of phosphatidylethanolamine.[1,2,3,4] The synthesis of lecithin in isolated enzyme systems proceeds by the following reactions:

CTP + phosphorylcholine ⟷ Cytidine diphosphate choline + inorganic pyrophosphate (1)

Cytidine diphosphate choline + D-α,β-diglyceride ⟷ Lecithin + CMP (2)

Enzyme preparations from chicken liver catalyze the net synthesis of lecithin when supplemented with cytidine diphosphate choline and D-α,β-diglyceride.[4] It also has been shown that D-α,β-diglycerides may be formed by the enzymatic dephosphorylation of phosphatidic acids.[4]

(1) E. P. Kennedy and S. B. Weiss, THIS JOURNAL, **77**, 250 (1955).
(2) E. P. Kennedy, *Can. J. Biochem. Physiol.*, **34**, 334 (1956).
(3) E. P. Kennedy and S. B. Weiss, *J. Biol. Chem.*, in press.
(4) S. B. Weiss, S. W. Smith and E. P. Kennedy, *Nature*, submitted

These results suggested that D-α,β-diglycerides might be common intermediates in the biosynthesis of phospholipides and of neutral fat. It has now been found that the same chicken liver enzyme preparations which carry out the synthesis of lecithin also catalyze the enzymatic net synthesis of triglyceride when supplemented with D-α,β-diglyceride and palmitoyl-S-Co A labeled with palmitic acid-1-C^{14} (Table I). Extensive incorporation of labeled palmitic acid into the triglyceride fraction was noted with the complete system. No significant incorporation was observed if the enzyme was previously boiled. Omission of D-α,β-diglyceride or substitution of equivalent amounts of palmitate + CoA for the palmitoyl-S-Co A reduced incorporation to low levels.

TABLE I

ENZYMATIC NET SYNTHESIS OF TRIGLYCERIDE

	Experimental conditions	Radioactivity of triglyceride fraction, total counts	Total triglyceride, micromoles
1	Complete system	210,000	2.46
2	Complete system, boiled enzyme	610	.72
3	D-α-β-Diglyceride omitted	12,200	.79
4	Palmitate + CoA in place of palmitoyl-S-Co A	3,350	.61

The complete system contained 1000 micromoles of Tris buffer of *p*H 7.4, 60 micromoles of $MgCl_2$, 40 micromoles of D-α,β-diglyceride derived from purified egg lecithin by treatment with lecithinase of *Cl. perfringens*,[5] 4 micromoles of palmitoyl-S-Co A labeled with palmitate-1-C^{14} (140,000 cts./micromole), 20 mg. of "Tween-20" (polyoxyethylene sorbitan monolaurate),[6] 80 micromoles of cysteine, and 1.0 ml. of a suspension of mitochondria from chicken liver in a final volume of 10 ml. The tubes were incubated for two hours at 40°. The lipides, after extraction from the enzyme system with ethanol, were taken up in carbon tetrachloride and repeatedly washed with aqueous ammoniacal ethanol to remove unreacted palmitate and palmitoyl-S-CoA. The triglyceride fraction was isolated from the lipid mixture by the chromatographic method of Borgström.[7] The radioactivity was measured by direct plating and counting of a small aliquot. The total triglycerides were estimated by the hydroxamic method.[8]

Chemical measurement of the total amount of triglyceride at the end of the experiment showed that the incorporation of radioactivity was not due to simple exchanges processes, since a considerable net increase of triglyceride was observed in the complete system, compared to the control with boiled enzyme.

These results support the conclusion that the synthesis of triglycerides in these preparations proceeds by the reaction:

D-α,β-diglyceride + palmitoyl-S-Co A ⟶ Triglyceride + CoA-SH (3)

A close interconnection is thus indicated between the enzymatic synthesis of phospholipides and of neutral fat.[9]

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(8) M. Rapport and N. Alonzo, *J. Biol. Chem.*, **217**, 193 (1955).
(9) The following abbreviations are used in this paper: CTP = cytidine-5, triphosphate; CMP = cytidine-5′ phosphate; CoA = coenzyme A