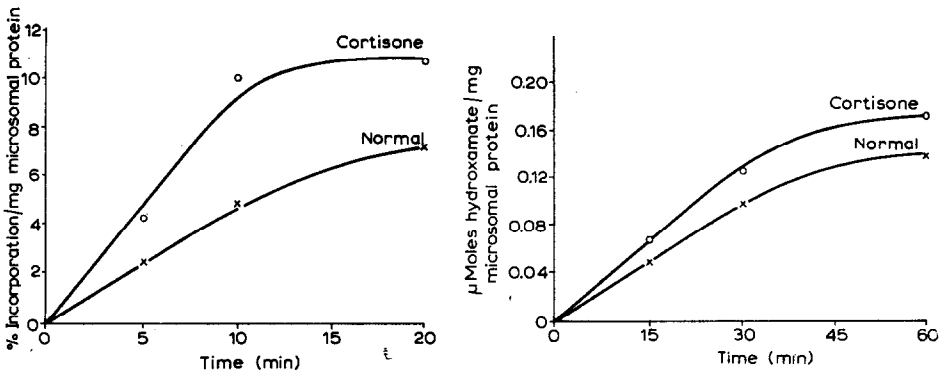

Fig. 1. Per cent incorporation of intravenous labeled palmitate into neutral fat by rat-liver microsomes. Adrenalectomized rats were fed and treated as in Table I. Four experiments, each utilizing a control and experimental rat, were performed: each point represents the average of four results.

Fig. 2. Micromoles of palmitoyl hydroxamate formed from palmitate by rat-liver microsomes. Adrenalectomized rats prepared as in Table I. Difference is significant at $P < 0.02$.

pool, but technical difficulties of measuring such small quantities have precluded any clear answer to this question.

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Department of Pathology, University of Colorado, School of Medicine, Denver, Colo. (U.S.A.)

ARTHUR P. HAYS
ROLLA B. HILL, JR.

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On the anabolism of lysolecithin

The conversion of lysolecithin into lecithin can be brought about by two different reactions:

lysolecithin + fatty acyl-CoA → lecithin (1)

and

2 lysolecithin → lecithin + glycerylphosphorylcholine (2)

Reaction 1 has been discovered by LANDS[1] and the enzyme system concerned has been detected in various animal tissues. Reaction 2 was first proposed by ERBLAND AND MARINETTI[2] to occur in a supernatant fraction from liver. In this system Reaction 1 may also contribute to the formation of lecithin. On the other hand our studies on a supernatant from bakers' yeast strongly suggested that Reaction 2 was exclusively responsible for the observed conversion of lysolecithin into lecithin[3]. However, because of an interference of phospholipase activity the two reaction products *viz.* lecithin and glycerylphosphorylcholine were not recovered in equimolar amounts.

By the use of doubly-labelled substrate confirmative evidence for Reaction 2 has now been obtained, and the relative quantitative importance of Reactions 1 and 2 was assessed in particle-free fractions from yeast and liver. When 1-[^{14}C]acylglycero-3-[^{32}P]phosphorylcholine having an isotopic ratio $^{14}C/^{32}P = A$ is converted in the presence of an excess of unlabelled fatty acids the lecithin formed either by Reaction 1 or 2 will exhibit an isotopic ratio of A or $2A$, respectively. The doubly-labelled substrate was prepared by phospholipase A (EC 3.1.1.4) hydrolysis of lecithin obtained from rat-liver homogenates incubated with radioactive phosphate and [1-^{14}C]palmitic acid[4].

The liver supernatant fraction was obtained after centrifugation of a homogenate of fresh tissue in Krebs–Ringer solution at $120000 \times g$ for 1 h. The yeast supernatant was prepared as described previously[3]. Aliquots of unlabelled fatty acid and 1-[^{14}C]acylglycero-3-[^{32}P]phosphorylcholine were mixed in solution and after removal of the solvent clear emulsions of the lipids in Krebs–Ringer were prepared by ultrasonic action. After addition of co-factors and enzyme system the mixtures were incubated at 37° under continuous agitation. The reaction was stopped by the addition of methanol–chloroform (2:1, v/v). Lipids were extracted according to the procedure of BLIGH AND DYER[5] and subjected to chromatography on silica-impregnated paper[6]. After staining with Rhodamine 6G the spots were cut out and counted for ^{32}P and ^{14}C activity with a Packard Tricarb liquid scintillation spectrometer. Quench corrections, usually differing not more than a few per cent for different vials, were made according to the method of HENDLER[7].

The extent to which lysolecithin was esterified and hydrolysed by the systems investigated is demonstrated by Table I.

TABLE I

CONVERSION OF LYSOLECITHIN INTO LECITHIN AND GLYCERYLPHOSPHORYLCHOLINE BY PARTICLE-FREE SUPERNATANTS OF YEAST AND LIVER

The incubation mixture consisted of 1 ml of supernatant, 10 μmoles of ATP, 0.2 μmole of CoA, 2 μmoles of linoleic acid and a small amount of labelled lysolecithin (about 0.2 μmole). After 1-h incubation at 37°, the lipids were separated and counted for ^{32}P activity. Values for liver are the mean ± S.D. of the mean of 9 experiments. For the yeast supernatant the values represent the mean of 6 experiments.

Supernatant fraction	*Distribution of radioactivity*		
	Deacylated water soluble products (%)	*Lysolecithin* (%)	*Lecithin* (%)
Yeast	91 ± 3	5.5 ± 3	3.5 ± 2.3
Liver	65 ± 10	14 ± 7	22 ± 11

The ratios of $^{14}C/^{32}P$ in the lecithin formed after incubation of lysolecithin with a yeast supernatant, without ATP and CoA present, turned out to be twice that of the lysolecithin substrate (Table II). The addition of ATP and CoA did not appear to give any significant decrease of this isotopic ratio, thus precluding a contribution of Reaction 1 to the synthesis of lecithin in this system. The results are in good

TABLE II

RATIO OF ^{14}C AND ^{32}P ACTIVITY OF LECITHIN FORMED FROM DOUBLY-LABELLED LYSOLECITHIN BY PARTICLE-FREE SUPERNATANTS FROM YEAST AND LIVER

The experimental conditions were the same as given in Table I.

Yeast				*Liver*			
$^{14}C/^{32}P$ ratio lysolecithin substrate (A)	*ATP and CoA*	*$^{14}C/^{32}P$ ratio lecithin (B)*	*B/A*	*$^{14}C/^{32}P$ ratio lysolecithin substrate (A)*	*ATP and CoA*	*$^{14}C/^{32}P$ ratio lecithin (B)*	*B/A*
0.24	—	0.51	2.11	0.24	—	0.43	1.79
2.20	—	4.44	2.02	5.70	—	7.45	1.31
2.20	+	5.00	2.27	2.20	—	4.86	2.20
5.70	+	12.5	2.19	5.70	+	6.95	1.22
0.40	+	0.84	2.10	0.40	+	0.57	1.42
0.99	+	1.89	1.91	0.99	+	1.45	1.47
1.21	+	2.43	2.01	1.21	+	1.56	1.29
				1.89	+	2.60	1.37
				2.80	+	3.68	1.31

agreement with our previous findings that, under the conditions used, exogenous fatty acids were not incorporated into lecithin[3]. Hence, it can be concluded that Reaction 2 is nearly completely responsible for the conversion of lysolecithin into lecithin in a yeast supernatant. In the particle-free liver fraction the lecithin formed in incubations without ATP and CoA also revealed an isotopic ratio $^{14}C/^{32}P$ nearly twice that of the lysolecithin substrate (Table II). It may be noticed that the data showed a considerable scattering for different enzyme preparations, but the values were found to be quite constant in duplicate experiments on one given liver supernatant. In agreement with the reports of ERBLAND AND MARINETTI[2,8] our findings are compatible with the occurrence of Reaction 2 in the liver system. Although even under these energy-poor conditions some incorporation of exogenous fatty acids according to Pathway 1 was found to occur, the formation of lecithin by Pathway 2 appears to be the more important reaction. However, in the liver supernatant the relative importance of the former Reaction 1 can be enhanced significantly by addition of ATP and CoA to the incubation mixture (Table II). It is worth noting that in a control experiment lysolecithin was converted by a liver homogenate in the presence of ATP and CoA into a lecithin having a ratio $^{14}C/^{32}P$ only 1.08 times that of the labelled lysolecithin. Apparently under these conditions Pathway 1 is by far the most important one in the liver homogenate.

Indications for the conversion of lysolecithin into lecithin according to Reaction 2 have been obtained in this laboratory not only in yeast and liver, but also in lung tissue, erythrocytes[4] and leucocytes[9]. It is of interest that erythrocyte ghosts were found to behave like the liver system, inasmuch as in both systems a direct acylation according to Reaction 1 occurs, particularly in the presence of ATP and CoA. By contrast, both in homogenates of leucocytes and in the particle-free yeast

system the conversion of lysolecithin into lecithin appears to involve Reaction 2 only. It may be of interest to assess whether this reaction is responsible for the occurrence of lecithins with two identical fatty acids *e.g.* dipalmitoleyl lecithin in yeast[10].

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Department of Biochemistry, H. VAN DEN BOSCH
The State University, H. A. BONTE
Utrecht (The Netherlands) L. L. M. VAN DEENEN

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