levels were maintained for a period of two weeks. The above results support the conclusion that a preferential incorporation of *protium* relative to *deuterium* is occurring simultaneously in these experiments and is probably of a magnitude comparable with the D:T factors reported here.[5]

TABLE I

Animal no.	Duration of expt.-days	Factor for preferential incorporation of deuterium[a] Liver glycogen	Liver fatty acids
1	3	1.06 ± 0.02	
2	4	1.08 ± .02	1.19 ± 0.02
3	2		1.17 ± .02
4	1	1.09 ± .02	1.19 ± .02

[a] This factor is defined as (T/D)water/(T/D)compound where T is proportional to the tritium atom fraction, D is the atom per cent excess of deuterium and the compound refers to glycogen or the fatty acid fraction. Normal deuterium abundance was taken to be 0.020 atom per cent.

Experimental.—Liver glycogen was prepared and purified following the procedure of Stetten and Boxer[6] while the method described by Schoenheimer and Rittenberg,[7] with minor modifications, was used to obtain the fatty acid fraction. Isotopic analyses were made on the hydrogen gas obtained by complete conversion over zinc at 415° of the water obtained by combustion. Memory effects were eliminated by measuring the results of a second and third combustion and conversion after discarding the products of a preliminary combustion intended to season the train. Deuterium was measured using a dual collector Nier-type hydrogen mass spectrometer while tritium was counted in the upper portion of the proportional region.[8] The reproducibility of the deuterium and tritium analyses is better than one per cent. The tritium atom fraction in the rat body fluid was approximately 10^{-10} while the deuterium concentration was kept below two per cent. in order to minimize the abundance of DOD molecules.

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RECEIVED DECEMBER 3, 1952

THE SYNTHESIS OF LECITHIN IN ISOLATED MITOCHONDRIA

Sir:

Recent reports from this laboratory[1,2] have shown that α-glycerophosphate is an important intermediate in the reaction scheme by which inorganic phosphate labeled with P^{32} is incorporated into the phosphorus-containing lipides of isolated rat liver mitochondria. Kornberg and Pricer[3,4] working with soluble enzyme extracts of rat liver have demonstrated the presence of enzymes capable of converting L-α-glycerophosphate into a lipide product tentatively identified as a phosphatidic acid. These workers[4] have also described an enzyme system which is capable of converting phosphorylcholine into a lipide product. With doubly-labeled phosphorylcholine (P^{32},C^{14}) the ratio of P^{32} to C^{14} in the product approximates that in the substrate, suggesting the incorporation of phosphorylcholine as a unit into a phospholipide molecule (presumably lecithin). Free choline is described by these authors as being only about one-tenth as active as phosphorylcholine in the formation of phospholipide.

It is the purpose of this communication to report the finding in isolated rat liver mitochondria of an enzyme system which incorporates free choline labeled with C^{14} into the lecithin fraction of the enzyme granules by a pathway which does not involve phosphorylcholine. When mitochondria isolated from sucrose homogenates of rat liver are incubated with choline-methyl-C^{14} and added cofactors, the mitochondrial phospholipides rapidly become radioactive. When phosphorylcholine-methyl-C^{14} of identical specific activity is tested in the same system, no significant incorporation of radioactivity into phospholipide is noted. Data from a typical experiment are shown in Table I. Similarly, P^{32}-labeled phosphorylcholine is also inactive as a precursor of radioactive phospholipide. If choline-methyl-C^{14} is tested in the presence of a large pool of unlabeled phosphorylcholine, no reduction in the radioactivity of the phospholipide fraction is observed. The lack of activity of phosphorylcholine in this system is not the result of the impermeability of the mitochondrial membrane to this substrate, since identical results are obtained with extracts of acetone powder preparations of mitochondria, which have been found to carry out

TABLE I

		Total radioactivity of phospholipides, cts./min.
	Experiment A	
1	Complete system	3000
2	"Zero time" control	68
3	Adenylic acid omitted	383
4	1.0 μM of 2,4-dinitrophenol added	759
5	Phosphorylcholine-methyl-C^{14} in place of choline-methyl-C^{14}	179
	Experiment B	
1	Complete system	1240
2	Phosphorylcholine-methyl-C^{14} in place of choline-methyl-C^{14}	52

In Experiment A, each vessel contained 15 μM of $MgCl_2$, 100 μM of sodium succinate, 3 μM of adenylic acid, 100 μM of phosphate buffer, *p*H 7.4 and 5.0 μM of choline-methyl-C^{14} or phosphorylcholine-methyl-C^{14} of identical specific activity (125,000 cts./μM/min.). The final volume was 3.0 ml. Approximately 20 mg. dry weight of freshly prepared rat liver mitochondria were added just prior to incubation for one hour in a Dubnoff apparatus at 38° with air as gas phase. The total phospholipide fraction was isolated and counted by methods closely similar to those described previously.[2] The complete system in Experiment B was exactly the same, except that the succinate and adenylic acid were replaced by 5.0 μM of adenosine triphosphate, and 1.0 ml. of a 10% extract of mitochondria acetone powder was used as enzyme instead of fresh mitochondria.

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(2) E. P. Kennedy, *J. Biol. Chem.*, in press.

(3) A. Kornberg and W. E. Pricer, Jr., THIS JOURNAL, **74**, 1617 (1952).

(4) A. Kornberg and W. E. Pricer, Jr., *Federation Proc.*, **11**, 242 (1952).

the incorporation of labeled choline into phospholipide when supplemented with ATP and other cofactors.

Choline-1,2-C^{14} and choline-methyl-C^{14} are incorporated at identical rates. The incorporation reaction is dependent upon oxidative phosphorylation for the generation of adenosine triphosphate in fresh preparations of mitochondria, being severely inhibited by the addition of dinitrophenol or the omission of adenine nucleotide. In acetone powder extracts, in which no oxidative phosphorylation occurs, there is an absolute requirement for adenosine triphosphate.

After hydrolysis of the phospholipide extracts with N/1 KOH at 37° by the method of Hack[5] the radioactivity may be quantitatively recovered as choline reineckate, indicating that it is lecithin rather than sphingomyelin which is labeled. By chromatography of the phospholipides by a variation of the method of Hanahan *et al.*,[6] radioactive lecithin fractions may be isolated with a choline/P ratio close to unity. The curves for the elution of C^{14} and lipide P from the columns are very nearly identical in these experiments.

The nature of the intermediates involved in the incorporation of choline into the lecithin of mitochondria, and in particular the possible role of phosphatidic acids in the process, is not yet known, and is the subject of continuing investigation in this laboratory.

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RECEIVED NOVEMBER 17, 1952

PEROXIDE- AND LIGHT-INDUCED REACTIONS OF ALCOHOLS WITH OLEFINS

Sir:

Preliminary results of a general program of research to determine if free-radical, chain addition is a property common to substances containing a methylene or methine group directly attached to an electronegative atom[1] have led to the discovery that primary and secondary alcohols add to olefins in the presence of peroxides or light. The reactions of ethanol, propanol-2, butanol-1 and butanol-2 with octene-1 in the presence of *t*-butyl peroxide give telemeric products whose chief components are, respectively, decanol-2, 2-methyldecanol-2, dodecanol-4 and 3-methylundecanol-3.

A reaction mixture containing ethanol (454 g., 9.87 moles), octene-1 (35 g., 0.31 mole) and *t*-butyl peroxide (4.4 g., 0.03 mole) was heated in a glass-lined, stainless-steel autoclave at 115–118° for 40 hours. Distillation, after the removal of *t*-butyl alcohol and unreacted ethanol, gave decanol-2 (11.6 g., b.p. 52–54° at 1 mm.; n^{20}D 1.4357; mol. wt. 152; m.p. of its α-naphthylurethane, 69°)[2,3], a product formed by the reaction of two molecules of octene-1 with one of ethanol (4.0 g., b.p. 120–126° at 1 mm.; n^{20}D 1.4479; mol. wt. 268), and a residue (18 g., mol. wt. 488).

In the apparatus described above a reaction mixture containing propanol-2 (356.7 g., 5.95 moles), octene-1 (28 g., .25 mole) and *t*-butyl peroxide (5 ml.) was held at 120° for 30 hours. Distillation gave *t*-butyl alcohol and unreacted propanol-2 (342.5 g., b.p. 81–82°), and a fraction shown to be 2-methyldecanol-2 (16.5 g., b.p. 49° at 0.1 mm.; n^{20}D 1.4359).

Anal. Calcd. for $C_{11}H_{24}O$: C, 76.67; H, 14.04; mol. wt., 172. Found: C, 76.35; H, 13.80; mol. wt., 178.

It was identical in infrared spectrum and other physical properties with 2-methyl-decanol-2 (b.p. 49.5° at 0.1 mm., n^{20}D 1.4358) prepared by the reaction of *n*-octylmagnesium bromide with acetone. Its allophanate (m.p. 113.5–114°; m.p. of mixture with authentic sample, 113–114°) was prepared.

Anal. Calcd. for $C_{13}H_{26}N_2O_3$: N, 10.8. Found: N, 10.5.

Further distillation gave a 2:1 product (7.4 g., b.p. 120–130° at 0.1 mm., mol. wt. 263) and a residue (7 g., mol. wt. 449). A similar reaction was observed when a mixture of propanol-2 (198.8 g., 3.31 mole) and octene-1 (19.9 g., 0.178 mole) was illuminated with a quartz mercury resonance lamp for 96 hours. 2-Methyldecanol-2 (6.0 g., b.p. 50–52° at 0.2 mm.; n^{20}D 1.4369; m.p. of its allophanate, 113–114°; m.p. of mixture with authentic sample, 113–114°), 2:1 product (3.1 g., n^{20}D 1.4525) and a residue (4.7 g., mol. wt. 422) were obtained.

The reaction of butanol-1 (581 g., 7.85 moles) with octene-1 (29.1 g., 0.26 mole) and *t*-butyl peroxide (3 g., 2 g. added after 18 hours) at 115–116° for 43 hours gave dodecanol-4 (18 g., b.p. 83–84° at 1 mm.; n^{20}D 1.4409).

Anal. Calcd. for $C_{12}H_{26}O$: C, 77.35; H, 14.07; mol. wt., 186. Found: C, 77.64; H, 13.82; mol. wt., 192.

This product was identical in infrared spectrum and other physical properties with dodecanol-4 (b.p. 83–84° at 1 mm., n^{20}D 1.4409) prepared by the reduction of dodecanone-4 (prepared by the peroxide induced reaction of *n*-butyraldehyde with octene-1)[4] with lithium aluminum hydride. Its α-naphthylurethane (m.p. 57–58°; m.p. of mixture with authentic sample, 57–58°) was prepared.

Anal. Calcd. for $C_{23}H_{33}NO_2$: C, 77.70; H, 9.36. Found: C, 77.53; H, 9.58.

A 2:1 product (7.5 g., b.p. 120–145° at 1 mm.; n^{20}D 1.4518, mol. wt. 297) and a residue (15 g., mol. wt. 421) were obtained.

Butanol-2 (247 g., 3.33 mole), octene-1 (26.5 g., 0.24 mole) and *t*-butyl peroxide (5 ml.) at 117–118° for 40 hours gave 3-methylundecanol-3 (13.3 g., b.p. 58–60° at 0.2 mm.; n^{20}D 1.4418).

Anal. Calcd. for $C_{12}H_{26}O$: C, 77.35; H, 14.07; mol. wt., 186. Found: C, 77.70; H, 14.18; mol. wt., 194.

It was compared as above with 3-methylundec-

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