TABLE III (*Continued*)

Substrate	Concn., $M \times 10^3$	Water, % by volume	Temp., °C.	Added reagent	Concn., $M \times 10^3$	k_1, sec.$^{-1}$	Run no.
Benzoyl fluoride	4.4	50	.5	.....	...	1.1×10^{-5}	156
	1.6	50	.5	H_3BO_3[a]	20	3.6×10^{-3}	149
				NaH_2BO_3	20		
	2.1	50	.5	H_3BO_3[a]	10	3.2×10^{-3}	151
				NaH_2BO_3	10		
Benzoyl bromide	3.7	50	.5	.....	...	6.3×10^{-2}	265
	4.8	50	.5	H_3BO_3[a]	20	5.3×10^{-2}	263
				NaH_2BO_3	20		
Benzenesulfonyl fluoride	5.1	50	25.1	.....	...	$<5 \times 10^{-3}$	257
	4.9	50	25.1	$HClO_4$	100	$<5 \times 10^{-3}$	260
	6.9	50	0.5	H_3BO_3[a]	20	1.8×10^{-5}	242
				NaH_2BO_3	20		

[a] Hydroxide ion concentration = 1.6×10^{-4} *N*.

Other reagents were analytical reagent grade or previously described.[4]

Procedure.—Most of the procedure has been described.[4] The rate of hydrolysis of acetyl fluoride in 25% water–75% acetone was determined by allowing a mixture of 150 ml. of acetone and 50 ml. of water to come to 25° in a 250-ml. polyethylene bottle and adding acetyl fluoride directly from a pipet. Aliquots (10 ml.) were shaken with 20 ml. of benzene, the aqueous layer removed, and the benzene extracted twice with 5 ml. of water. The water solutions were combined and titrated for fluoride ion.

The hydrolysis of acetyl fluoride in 50% water–50% acetone was accomplished by cooling a mixture of 45 ml. of acetone and 50 ml. of water at 0.5° in the 100-ml. round-bottomed reaction cell and adding the acetyl fluoride in 5 ml. of cold acetone. The 10-ml. aliquots were shaken with 20 ml. of chloroform and titrated for fluoride ion. When an inert salt or an acid was present, 5 ml. of 2 *N* lithium perchlorate or perchloric acid replaced 5 ml. of water in the solvent.

The hydrolyses of benzoyl fluoride and benzenesulfonyl fluoride were followed in a similar manner. The reaction cell was a 250-ml. polyethylene bottle and the solvent was 100 ml. of acetone and 100 ml. of water. The aliquots for benzoyl fluoride were 20 ml., those for benzenesulfonyl fluoride were 10 ml. Since benzenesulfonyl fluoride hydrolyzed at an extremely slow rate, if at all, the 100% point was found by hydrolyzing a 10-ml. aliquot with sodium hydroxide and titrating for fluoride ion. The reaction proceeded to less than 10% in 2.2×10^6 seconds (26 days). The presence of 0.1 *N* lithium perchlorate or perchloric acid had no apparent effect on the rate.

Table III gives supporting kinetic data in addition to those previously reported.[4]

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COMMUNICATIONS TO THE EDITOR

THE FRACTIONATION OF HYDROGEN ISOTOPES IN BIOLOGICAL SYSTEMS[1]

Sir:

Although deuterium has been extensively used as an isotopic tracer in studies of intermediary metabolism,[2] relatively little is known about the H:D fractionation that occurred and its effect on the quantitative interpretation of the metabolic data. Although this factor can be measured in chemical reactions it is inherently difficult to measure in metabolic (*in vivo*) studies utilizing only protium (H) and deuterium. However, the use of precursor compounds labeled with both deuterium and tritium can yield precise values for D:T fractionation effects in such studies and the latter can then be used to estimate these effects for D relative to H.[3] We have administered water containing D and T to rats by intraperitoneal injection in order to bring the deuterium body water level up to about two per cent. and then supplied drinking water having the same T/D ratio for several days to maintain this level. Analysis of the glycogen and fatty acid fractions from the livers of these animals shows a preferential incorporation of the deuterium by approximately 8 and 18 per cent., respectively (Table I). The results for the fatty acids are in qualitative agreement with those recently reported by Glascock and Dunscombe.[4] In the latter experiments, the body fluid isotope

(1) This work was supported in part by grants-in-aid from the Atomic Energy Commission No. AT(30-1)-910.

(2) R. Schoenheimer, Dynamic State of Body Constituents, Harvard University Press, 1946; M. Kamen, Radioactive Tracers in Biology, Chap. VII, Academic Press, N. Y., 1951.

(3) W. G. Verley, J. R. Rachele, V. du Vigneaud, M. L. Eidinoff and J. E. Knoll, THIS JOURNAL, **74**, 5941 (1952). When methanol containing CD_3OH, CHD_2OH, CH_2DOH and CH_2TOH was administered to rats, the (T/D) ratio in the methyl groups of choline and creatine was greater than the corresponding ratio in the administered methanol.

(4) R. F. Glascock and W. G. Dunscombe, *Biochem. J.*, **51**, August, (1952), xl, Communication to Proceedings of the Biochemical Society.

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.

(5) J. Bigeleisen, *Science*, **110**, 14 (1949).

(6) D. W. Stetten, Jr., and G. E. Boxer, *J. Biol. Chem.*, **155**, 231 (1944).

(7) R. Schoenheimer and D. Rittenberg, *ibid.*, **111**, 177 (1935).

(8) M. L. Eidinoff, J. E. Knoll, D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **74**, 5280 (1952).

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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.

(1) E. P. Kennedy, *Federation Proc.*, **11**, 239 (1952).

(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).