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	.....	...	$<5 \times 10^{-8}$	257
	4.9	50	25.1	$HClO_4$	100	$<5 \times 10^{-8}$	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_3TOH 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).

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.

(5) M. H. Hack, *J. Biol. Chem.*, **169**, 137 (1947).

(6) D. J. Hanahan, M. B. Turner and M. E. Jayko, *ibid.*, **192**, 623 (1951).

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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-

(1) *Cf.* W. H. Urry, O. O. Juveland and F. W. Stacey, THIS JOURNAL, **74**, 6155 (1952).

(2) D. W. Adamson and J. Kenner, *J. Chem. Soc.*, 842 (1934).

(3) R. H. Pickard and J. Kenyon, *ibid.*, **99**, 55 (1911).

(4) M. S. Kharasch, W. H. Urry and B. M. Kuderna, *J. Org. Chem.*, **14**, 248 (1949).

anol-3 (b.p. 63° at 0.25 mm., $n^{20}D$ 1.4416) prepared from *n*-octylmagnesium bromide and butanone-2. Its allophanate (m.p. 76–77°; m.p. of mixture with authentic sample, 76–77°) was made.

Anal. Calcd. for $C_{14}H_{28}N_2O_3$: N, 10.29. Found: N, 10.44.

2:1 Product (6.2 g., b.p. 150–160° at 0.2 mm.; $n^{20}D$ 1.4552; mol. wt. 311) and a residue (10.4 g., mol. wt. 518) were obtained. This work is continuing.

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RECEIVED DECEMBER 15, 1952

BOOK REVIEWS

Chemistry of Carbon Compounds. Volume I, Part A. General Introduction and Aliphatic Compounds. By E. H. RODD (Editor), A.C.G.I., D.I.C., D.Sc., F.R.I.C. Elsevier Publishing Company, 402 Lovett Boulevard, Houston, Texas, 1951. xxv + pages 1–778. 16.5 × 23 cm. Subscription price, $18.00. List price 15% higher.

The appearance of a publication of the scope and magnitude of "Chemistry of the Carbon Compounds," the initial volume of which is now available, represents a landmark in the history of chemical publishing.

Growing out of a clearly-recognized need for revision of Richter's "Organic Chemistry," the book maintains the general organization of its predecessor but is in every other way an entirely new effort. A group of distinguished advisors, headed by Sir Robert Robinson and including J. W. Cook, R. D. Haworth, Sir Ian Heilbron, E. L. Hirst and A. R. Todd, have presumably aided Editor E. H. Rodd in the organization of the work and the selection of authors, twenty-three of whom have contributed to this first volume.

The general organization of the work calls for five volumes as follows: I, General Introduction, Aliphatic Compounds; II, Alicyclic Compounds; III, Aromatic Compounds; IV, Heterocyclic Compounds; V, Miscellaneous.

This first book, part A of Volume I, is devoted to a general introduction and a survey of aliphatic compounds up to and including dicarbonyl compounds. The former gives short accounts of the history of organic structural chemistry, classification and nomenclature, and the literature of organic chemistry, as well as a more detailed discussion of analytical methods. Chapters on physical properties, crystallography, light absorption, the concept of acids and bases, stereochemistry, reaction mechanisms and free radical reactions complete the introduction. Although certain of these chapters are excellent, some are too short to be of real value; they serve merely to acquaint the reader with a few of the more important ideas and to provide him with a selected bibliography for further reading. Perhaps this reflects the intentions of the Editor and his Advisory Board; in justice it must be said that for adequate treatment some of the topics would require much more space than could be allocated in this work.

It is impossible to review adequately the remainder of the first volume on such short acquaintance. For one to acquire the intimate acquaintance necessary for authoritative comment, the book would have to remain at hand over a period of years, referred to continually and read occasionally in systematic fashion. All that can be said now is that a superficial scrutiny shows excellent organization and adequate coverage for the sections examined.

One is at first tempted to compare this work with its predecessor, Richter, and with that remarkable work of another generation, Meyer-Jacobson. In this reviewer's opinion, the latter has never been equalled, and in view of the enormously increased scope of the science, probably cannot be. It is this very increase in scope which renders such a comparison meaningless. The Editors, Advisors and Authors of the present work faced a different and vastly more complex task than those of the earlier works, and are to be congratulated for the assumption of so formidable a burden.

The series is certain to become a standard reference work available in all libraries and occupying a position in the personal libraries of many organic chemists. The appearance of subsequent volumes will be awaited with interest.

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MARSHALL GATES

The Alkaloids—Chemistry and Physiology. Volume II. By R. H. F. MANSKE, Dominion Rubber Research Laboratory, Guelph, Ontario, and H. L. HOLMES, Riker Laboratories, Inc., Los Angeles, California (Editors). Academic Press, Inc., 125 East 23rd Street, New York 10, N. Y. 1952. viii + 587 pp. 16.5 × 23.5 cm. Price, $14.50.

The first volume of this series was received with enthusiasm by organic chemists. The present work is equally deserving. It is the expressed intent of the editors "to present a readable and comprehensive work which will include all matters of importance in alkaloid chemistry." For the most part, this aim has been achieved very successfully.

This is a timely monograph. A high proportion of the systems discussed are under intensive study at present. The list of chapter headings and authors include: Morphine I (H. L. Holmes), Morphine II (H. L. Holmes and G. Stork), Colchicine (J. W. Cook and J. D. Loudon), Alkaloids of the Amaryllidaceae (J. W. Cook and J. D. Loudon), The Acridine Alkaloids (J. R. Price), The Erythrina Alkaloids (L. Marion), The Strychnos Alkaloids II (H. L. Holmes).

The chapters are not of equal quality. The presentation of the stereochemistry of the morphine family deserves special praise; however, other sections concerning these alkaloids are at times both tedious and obscure. The section on colchicine makes fascinating reading. The biological effects of this substance are discussed in some detail—a subject largely neglected in the other chapters.

The discussion of the indole group is divided into three main sections: indole, Erythrina and Strychnos alkaloids. The chapter entitled "The Indole Alkaloids" contains the most complete discussion of these bases presently available. A number of ill-defined bases are included, presumably on a biogenetic basis, where the evidence for the presence of an indole system appears based on intuition rather than fact. Extensive recent developments in the chemistry of the Erythrina bases make that chapter seriously out of date. In the final chapter of the indole sequence, developments in the strychnine field which have appeared since the publication of Volume I are discussed.

The editors and authors of this series are to be complimented on the fine job that they are doing. They are filling real need. That some portions of these volumes are out of date at the time of publication should not distress them.