TABLE I

In addition to 1 μmole of the appropriate acyl CoA derivative each tube contained (in μmoles): Tris, *p*H 8.5, 100; ATP, 4; $MgCl_2$, 4; glutathione, 5; $KHC^{14}O_3$ (specific activity, 47,000 c.p.m. per μmole), 15 and enzyme. 1.02 and 0.20 units of purified carboxylase (see text) were added to tubes containing acetyl- and propionyl-CoA, respectively. Final volume was 1.5 ml.; 20-minute incubation at 37°.

Additions	$HC^{14}O_3^-$ fixed per hour per mg. of protein[4] (μMoles)
None	0.0
Acetyl CoA	0.3
	0.4
Propionyl CoA	35.2
	33.3
Butyryl CoA	1.7
	1.6

hours. The concentrated ether extract was chromatographed (ascending) on Whatman No. 3MM paper using the isoamyl alcohol saturated with 4 *N* formic acid solvent system.[3] The single radioactive spot observed (R_f 0.85) was identified tentatively as ethylmalonic acid (R_f 0.85). The hydroxamate of the radioactive reaction product was prepared after conducting the enzymatic carboxylation reaction as described above. At the end of the incubation 1000 μmoles of neutralized hydroxylamine was added and after a 30-minute period of 0° the hydroxamates were extracted[10] and paper chromatographed as described above. A single radioactive spot (R_f 0.53) which also gave a purple color when sprayed with ferric chloride reagent was observed. Authentic ethylmalonyl monohydroxamate exhibited an R_f value (0.53) identical to that of the hydroxamate of the labeled reaction product. These results indicate that ethylmalonyl CoA is the product of the enzymatic carboxylation of butyryl CoA.

(10) E. R. Stadtman and H. A. Barker, *J. Biol. Chem.*, **184**, 769 (1950).

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RECEIVED OCTOBER 21, 1959

BIOLOGICAL CHLORINATION. IV. PEROXIDATIVE NATURE OF ENZYMATIC CHLORINATION[1]

Sir:

We have reported the extraction of a soluble β-ketoadipate chlorinase system from mycelial powders of *Caldariomyces fumago* which catalyzes the formation of δ-chlorolevulinic acid from β-ketoadipic acid and chloride ion.[2-4] Purification of these crude extracts revealed a multiple enzyme requirement and a heat stable cofactor requirement for enzymatic chlorination. As shown in Table I, after calcium phosphate gel treatment of the crude extract, a heat stable supernatant fraction and a heat labile gel eluate fraction are required for the formation of δ-chlorolevulinate.

(1) This work was supported in part by a grant (No. G-6403) from the National Science Foundation.

(2) P. D. Shaw and L. P. Hager, THIS JOURNAL, **81**, 1011 (1959).

(3) P. D. Shaw, J. R. Beckwith and L. P. Hager, *J. Biol. Chem.*, **234**, 2560 (1959).

(4) P. D. Shaw and L. P. Hager, *ibid.*, **234**, 2565 (1959).

TABLE I

HEAT STABLE FACTOR REQUIREMENT FOR β-KETOADIPATE CHLORINASE SYSTEM

The complete system contained 200 μmoles of potassium phosphate buffer, *p*H 4.8, 10 μmoles of KCl^{36} at a specific activity of 9,700 c.p.m. per μmole, 20 μmoles of potassium β-ketoadipate plus the additions listed below in a total volume of 1 ml. After 1 hour of incubation at 30° under aerobic conditions the reaction was stopped by the addition of 0.2 ml. of 7 *N* sulfuric acid. The formation of δ-chlorolevulinic acid was determined as described previously.[4]

	Additions	δ-Chlorolevulinate synthesis, mμmoles
1	0.9 mg. calcium phosphate gel eluate	7
2	4.5 mg. calcium phosphate gel supernatant	11
3	1 + 2	52
4	1 + heated supernatant	31
5	2 + heated eluate	6
6	1 + acid hydrolyzed supernatant	690
7	1 + 100 μmoles glucose	2,834
8	1 + 7 μmoles H_2O_2	1,200

Mild acid hydrolysis (0.1 *N* H_2SO_4, 1 hour, 120°) markedly enhances the activity of the heat stable fraction and active extracts contain large amounts of a polysaccharide[4] (glucose polymer). The hypothesis that the polysaccharide is the active factor is supported by the observation that glucose will replace the requirement for the heat stable fraction. Hydrogen peroxide also is active in replacing the heat stable fraction, indicative that the polysaccharide and its hydrolytic product, glucose, function in a hydrogen peroxide generating system. This has been established further by separation of the heat labile gel–eluate fraction into two enzymatic components on diethylaminoethyl cellulose columns. As shown in Table II both a glucose oxidase fraction plus a chloroperoxidase fraction are required for δ-chlorolevulinic acid formation from β-ketoadipic acid, chloride ion and glucose.

TABLE II

REQUIREMENT FOR HYDROGEN PEROXIDE GENERATING SYSTEM

The conditions are the same as those described in Table I.

	Additions	δ-Chlorolevulinic acid synthesis mμmoles
1	Complete (2 through 6)	251
2	Complete minus 7 γ chloroperoxidase	13
3	Complete minus 14 γ glucose oxidase	28
4	Complete minus 100 μmoles glucose	4
5	Complete minus 20 μmoles β-ketoadipate	14
6	Complete minus 10 μmoles KCl	0

The enzymatic reactions leading to the formation of δ-chlorolevulinic acid in the crude extracts which contain catalase may therefore be formulated:

$$(\text{glucose})_n + 3H_2O \xrightarrow{\text{hydrolase}} (\text{glucose})_{n-3} + 3\,\text{glucose} \quad (1)$$

$$3\,\text{glucose} + 3O_2 + 3H_2O \xrightarrow[\text{oxidase}]{\text{glucose}} 3\,\text{gluconic acid} + 3H_2O_2 + 3H^+ \quad (2)$$

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 1\ O_2 + 2\ H_2O \qquad (3)$$

$$1\ H_2O_2 + Cl^- + {}^-OOC\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}CH_2\text{-}COO^- + 2\ H^+ \longrightarrow CO_2 + Cl\ CH_2\text{-}CO\text{-}CH_2\text{-}CH_2\text{-}COO^- + 2\ H_2O \qquad (4)$$

Sum:

$$3\ \text{glucose} + 2O_2 + Cl^- + {}^-OOC\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}CH_2\text{-}COO^- \longrightarrow 1\ CO_2 + 3\ \text{gluconic acid} + ClCH_2\text{-}CO\text{-}CH_2\text{-}CH_2\text{-}COO^- + H_2O + H^+ \qquad (5)$$

(5) Postdoctorate Research Fellow of the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

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RECEIVED OCTOBER 28, 1959

BOOK REVIEWS

Gmelin's Handbuch der Anorganischen Chemie. Achte Völlig Neu Bearbeitete Auflage. **Sauerstoff.** Lieferung 3. System-Nummer 3. E. H. ERICH PIETSCH, Editor. Verlag Chemie, G.m.b.H., (17a) Weinheim/Bergstr., Pappelallee 3, Germany. 1958. xi + 518 pp. 17.5 × 25.5 cm. Price, DM 283.--.

The volume concerns primarily the properties of elementary oxygen. No compounds are discussed other than in relation to their influence on the properties of or their use in the preparation of oxygen.

The principal topics covered are: the preparation of the element; physical properties of the oxygen molecule, oxygen atom and the oxide ion; molecular properties and electronic structures of species such as O_2, O_2^+, O_2^- and O_4; physical and thermodynamic properties of oxygen; spectral properties of oxygen; electrochemical properties of oxygen; the reaction between oxygen and hydrogen. Little or no discussion of ozone or water is given in this volume.

Of particular note are the extensive references concerning the theoretical interpretation of the properties discussed.

The reaction between hydrogen and oxygen is treated in great detail and with considerable reference to the mechanisms of these reactions.

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Radioisotopes in Scientific Research. Proceedings of the International Conference held in Paris in September, 1957, under the auspices of the United Nations Educational Scientific and Cultural Organization. Volume II. Research with Radioisotopes in Chemistry and Geology. Edited by R. C. EXTERMANN, Institut de Physique Expérimentale, Université de Gènève. Pergamon Press, Inc., 122 East 55th Street, New York 22, N. Y. 1958. xxi + 741 pp. 17.5 × 25.5 cm. Price, $22.50. Complete set of four volumes, $80.00.

This volume is one of four that comprise the collected papers presented at the First (UNESCO) International Conference on Radioisotopes in Scientific Research. The meeting was held in Paris in September, 1957. The other three volumes deal with the use of radioisotopes in "Physics and Industry," "Human and Animal Biology," and "Plant Biology and some General Problems."

Volume II presents 53 papers that were delivered at 11 separate sessions of the conference. These meetings dealt with organic chemistry (2 sessions), recoil chemistry, analytical chemistry (2 sessions), physical chemistry (3 sessions), geophysics (2 sessions) and the production of radioisotopes. Forty of the papers are presented in English, the other 13 in French. All of the papers are accompanied by abstracts in English, French, Spanish and Russian. The papers are usually followed by a brief "discussion," *i.e.*, questions and answers.

The papers presented in this volume are quite diverse and deal individually only with rather specific scientific problems. However, a reading of the papers in those areas of research with which this reviewer is most familiar (organic chemistry and radiation chemistry) has given an impression of high quality. The organic chemistry papers are almost exclusively concerned with mechanism problems, and they are further illustrations of the power of the tracer method in solving these problems. The radiation chemistry papers are all concerned with the subject of recoil chemistry—excellent summaries of studies of the effects on organic compounds of recoiling tritium or carbon atoms are presented. The three physical chemistry sessions covered a great variety of topics. As examples of some of the subjects covered may be mentioned electron exchange reactions of iron and cobalt complexes, the exchange of Cl^{36} between HCl and CH_3Cl on tungsten films at 150 and 300°, chemical dosimetry, diffusion of water vapor through polymer membranes, and solvent-extraction studies of metal halides at low concentrations.

The book shows the effects of a very-hasty assembling, and the price is high. It does, however, serve a very useful purpose in presenting the record of an excellent conference on research using radiotracers.

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Russian for the Scientist. By JOHN TURKEVICH, Ph.D., Eugene Higgins Professor of Chemistry, Princeton University, and LUDMILLA B. TURKEVICH, Ph.D., Lecturer in Russian, Princeton University. D. Van Nostrand Co., Inc., 120 Alexander Street, Princeton, N. J. 1959. ix + 255 pp. 16 × 23.5 cm. Price, $5.95.

The growing literature of English–Russian grammars, dictionaries, and handbooks has a worthy addition in this recent book by Professor and Mrs. Turkevich. The authors' aim has been to furnish the scientist "with a tool with which he may go to Russian scientific literature and extract the particular information he seeks. . . ." The "tool" is comprised of twenty lessons, plus appendixes and glossaries. While presumably the book is intended for use in formal classroom instruction, the lessons are nevertheless sufficiently concise and clear for use by a determined do-it-yourself devotee.

The student is introduced first to the Russian alphabet and the pronunciation of Russian words. From the beginning emphasis is placed on cognates and their use in building vocabulary. Subsequent lessons treat nouns and their cases, pronouns, verbs and their aspects and moods, and much of the other minutiae of which grammar is composed. Excerpts from Russian scientific texts are introduced with the more advanced lessons. These selections, ranging up to about 200 words in length, are from the fields of aeronautical engineering, biology, chemistry and physics, with emphasis on chemistry. While mastery of grammar is secondary to the principal objective of providing a tool for the scientist, there is no hint of a "twenty easy lesson" approach to learning Russian. The fundamentals are all there, and the survivor of a tour through the 200-odd pages of this book will be well repaid for his efforts.

The book is well printed, and the reviewer found the illustrations neatly done and to the point.

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