rise in non-protein nitrogen (NPN) and the increase in % of radioactivity in the TCA supernatant. This observation is presumptive evidence that insulin was being proteolytically degraded by the extract and that the determination of % of radioactivity in the TCA supernatant is a valid method for detection of this proteolysis.

In order to relate proteolysis with the inactivation of the biological action of insulin, the radioactive insulin assay was compared directly with the rabbit hypoglycemia test of Mirsky and Broh-Kahn.[3] In this case, each of the incubation mixtures was divided into two parts and assayed by both methods. With increased proteolysis, decreased biological activity of the incubated insulin was demonstrated. As a further comparison, studies of various inhibitors with known effects on the insulin-inactivating action of liver extract were repeated. In these cases, only the assay for radioactivity was carried out. The results with these inhibitors were in good agreement with the earlier work in which the rabbit assay was used.[3]

Studies on the specificity of the enzyme system have been made. The results of an experiment using the technique of substrate competition are shown in Fig. 2. Although amorphous insulin in excess clearly depressed the rate of degradation of insulin-I^{131} in a given amount of extract, the addition of the same weight of α-lactalbumin, human serum albumin or casein had negligible effects. This indicated that of these four proteins, only amorphous insulin effectively competed with insulin-I^{131} in this system. In order to substantiate this view, these four proteins were incubated with liver extract for 30 min. and increases in NPN were determined. Only with insulin as substrate was there a measurable increase in NPN over the control incubation mixture. These experiments demonstrate that the insulin-inactivating enzyme system has some degree of specificity.

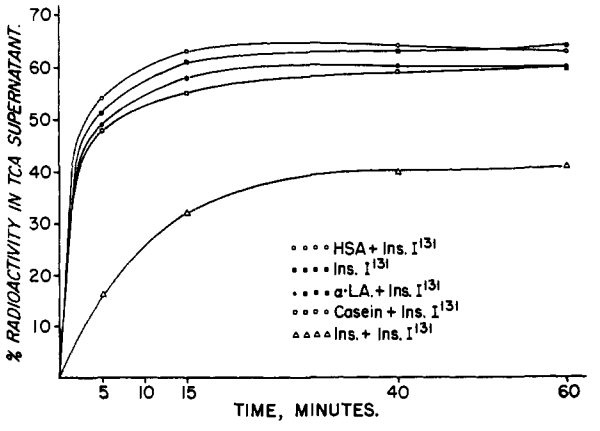

Fig. 2.—Changes with time in % radioactivity in TCA supernatant: Incubation of tracer amount of insulin-I^{131} alone and with 0.125 mg. of specified proteins; Extract used obtained from 0.3 g. rat liver.

DEPARTMENT OF MEDICINE
SCHOOL OF MEDICINE
UNIVERSITY OF WASHINGTON
SEATTLE 2, WASHINGTON

H. H. TOMIZAWA
M. L. NUTLEY
H. T. NARAHARA
R. H. WILLIAMS

RECEIVED MAY 13, 1954

MECHANISM OF ENZYMATIC CARBON DIOXIDE FIXATION INTO OXALOACETATE

Sir:

This report concerns the mechanism of the enzymatic conversion of phosphoenol pyruvate (PEP) and carbon dioxide into oxaloacetate (OAA) and inorganic phosphate. Such a reaction was first obtained with spinach leaf preparations by Bandurski and Greiner.[1] The present studies were made with dialyzed extracts of wheat germ. By conducting the reaction in a medium of D_2O, it has been possible to show that it is the keto form of oxaloacetate, (and not an enol) which results from the carbon dioxide fixation reaction. The mechanism is therefore similar to the reverse of the mechanism demonstrated by Steinberger and Westheimer[2] for the decarboxylation of dimethyloxaloacetate. Our results also permit the conclusion that if a phosphorylated derivative of OAA is formed as an intermediate in the reaction, the phosphate must be attached to a carboxyl group.

The wheat germ extracts contain a very active malic dehydrogenase as well as the enzymes necessary for the conversion of 3-phosphoglyceric acid (PGA) to PEP. Fumarase is absent, however. It was possible, therefore, to form malate from PGA and carbon dioxide by incubation of the latter two substances with wheat germ extract and reduced diphosphopyridine nucleotide (DPNH), generated with ethanol and alcohol dehydrogenase. Since the OAA is reduced as soon as it is formed, there is little opportunity for non-enzymatic keto-enol tautomerization.

This reaction was carried out in a medium of D_2O, and the malate was isolated as the diphenacyl ester and analyzed for D. In two separate experiments, the malate was found to contain 0.10 and 0.05 atoms of D per molecule. This shows that no enol form of OAA (or a derivative thereof) is a necessary intermediate in the reaction sequence, since, if such were the case, the malate should contain a minimum of one atom of D per molecule. The results also show that malic dehydrogenase causes a direct transfer of hydrogen from DPNH to the carbonyl carbon atom of OAA. The reactions may be formulated by equations 1 through 4:

$$\begin{aligned} &CH_2OPO_3^{=}CHOHCOO^- \rightleftharpoons \\ &CH_2OHCHOPO_3^{=}COO^- \rightleftharpoons CH_2{=}COPO_3^{=}COO^- \quad (1) \\ &CH_2{=}COPO_3^{=}COO^- + CO_2 + OH^- \longrightarrow \\ &\qquad COO^-CH_2COCOO^- + HPO_4^{=} \quad (2) \\ &COO^-CH_2COCOO^- + DPNH + H^+ \rightleftharpoons \\ &\qquad COO^-CH_2CHOHCOO^- + DPN^+ \quad (3) \\ &CH_3CH_2OH + DPN^+ \rightleftharpoons CH_3CHO + DPNH + H^+ \\ &\qquad (4) \end{aligned}$$

The experiments were carried out as follows: 130 μM. PGA, 160 μM. $MgSO_4$, 200 μM. phosphate buffer of *p*H 7.0, 1 ml. ethanol, 4 μM. DPNH, 1 μg. crystalline yeast alcohol dehydrogenase, 2.0 ml. of dialyzed wheat germ extract[3] and 18 ml. of 95%

(1) R. S. Bandurski and C. M. Greiner, *J. Biol. Chem.* **204**, 781 (1953).

(2) R. Steinberger and F. H. Westheimer, THIS JOURNAL, **73**, 429 (1951).

(3) R. C. Barnett, H. A. Stafford, E. E. Conn and B. Vennesland, *Plant Physiol.*, **28**, 115 (1953).

D_2O were incubated at room temperature for 60 minutes. The reaction was stopped by addition of sulfuric acid and the L-malic acid was extracted with ether. Analysis showed that 13.3 μM. of malate had been formed. After suitable dilution, a sample of the malate was converted to the diphenacyl derivative. The procedure was similar to that previously described for the preparation of phenacyl lactate.[4]

Separate experiments with OAA and DPNH in D_2O confirmed the fact that malic dehydrogenase catalyzes a direct transfer of hydrogen between coenzyme and the keto form of OAA. These results are consistent with previous studies of other pyridine nucleotide dehydrogenases.[5] In addition, experiments with enzymatically reduced DPND (reduced monodeuterio DPN) showed that malic dehydrogenase uses the same side of the nicotinamide ring as do alcohol dehydrogenase and lactic dehydrogenase.[4,5,6] These experiments will be described in detail elsewhere.

This investigation was supported in part from grants from the National Institutes of Health, United States Public Health Service, and by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago and the American Cancer Society on recommendation of the Committee on Growth.

(4) F. A. Loewus, P. Ofner, H. F. Fisher, F. H. Westheimer and B. Vennesland, *J. Biol. Chem.*, **202**, 699 (1953).

(5) B. Vennesland, F. H. Westheimer, "The Mechanism of Enzyme Action," W. D. McElroy, Johns Hopkins Press, Baltimore, Md., 1954, p. 307.

(6) H. F. Fisher, E. E. Conn, B. Vennesland and F. H. Westheimer, *J. Biol. Chem.*, **202**, 687 (1953).

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CHICAGO
CHICAGO, ILL.

BIRGIT VENNESLAND
T. T. TCHEN
FRANK A LOEWUS

RECEIVED MAY 6, 1954

BOOK REVIEWS

Chemistry of the Lanthanons. By R. C. VICKERY, D.Sc., Ph.D. Academic Press, Inc., 125 East 23rd Street, New York 10, N. Y. 1953. viii + 296 pp. 15 × 22 cm. Price, $6.00.

Dr. Vickery defines the lanthanons as "that series of elements whose atomic numbers range from 57 to 71 inclusive and whose properties endow them with a location in the sixth period of Group IIIA of the Periodic System." Most chemists probably still refer to these elements as the "rare earths," but this archaic and misleading designation suggests no logical terminology for the analogous series which includes the heavy synthetic elements.

In 1947, J. K. Marsh proposed that the two series be designated "lanthanons" and "actinons," respectively, but American chemists, whose etymological sense perhaps does not match that of their English colleagues, have rather generally adopted the rival terms "lanthanides" and "actinides." At the risk of being accused of chauvinism, the reviewer will hereafter use "lanthanides" in place of "lanthanons."

The increasing availability of these elements in pure form at reasonable cost, and a growing appreciation of their unique value in fundamental investigations into the physics and chemistry of both solids and solutions, suggest that they will continue to be of interest to physical and inorganic chemists for some years to come.

"The Chemistry of the Lanthanons" may be expected, therefore, to attract the attention of a much wider audience than the small band of veteran "rare earth chemists." In attempting to assess the merits of Dr. Vickery's book it is essential to recognize that it was written at a time when ion exchange techniques were effecting revolutionary advances in methods of separating and purifying the lanthanides, but before these methods were widely adopted. (Literature abstraction for the book is said to be complete through December, 1951, but references to some important papers of earlier date are missing.) As a result, the sections on separation and purification, which comprise about half of the text, describe many methods which ion exchange techniques have now rendered obsolete. The few pages devoted to exchange methods are inadequate to represent their present importance. This deficiency is compensated in part by the very thorough discussion of methods of separation based on oxidation or reduction processes.

The remaining chapters are as follows: 1, Historical; 2, Modes of Occurrence; 3, Structure, Spectroscopy and Paramagnetism; 4, Isotopic Composition, Radioactivity, and Valency; 13, Properties of the Lighter Lanthanons; 14, Properties of the Heavy Lanthanons; 15, Analytical Methods; 16, Uses and Applications of the Lanthanons. The chemistry of yttrium is summarized in a four-page appendix.

The first two chapters are excellent. A fascinating, if brief, account of the work of the pioneers in "rare earth" chemistry is followed by a thorough and satisfying discussion of the distribution, geochemistry and mineralogy of the lanthanides.

In the next chapter, however, Dr. Vickery evidently is on unfamiliar ground for his discussion of electronic structure, spectroscopy and paramagnetism is marred by errors of fact as well as by occasional sentences which seem to defy comprehension. As an example of a factual error, in discussing the absorption spectrum of cerous ion he has confused the spectroscopist's notation CeIV with the chemist's Ce^{4+} ion, while the inquiry "Is it possible that the subsidiary bands to the violet side of the strong sharp bands are but umbra and penumbra effects due to scattering by alien ions and/or reflections from the walls of defects produced in the atom structure by the gradual variation in the contents of the 4f and 5d shells?" still loses the reviewer at about midsentence.

Similar criticisms apply elsewhere in this chapter, which can hardly be regarded as a sound introduction to the subject matter covered.

Isotopic composition and radioactivity are considered in the fourth chapter, along with the unrelated subject of valency. The former are dealt with chiefly by a table of isotopes giving abundances, half-lives and modes of decay. The discussion of valency makes little use of thermodynamic reasoning, the non-tripositive states being ascribed qualitatively to the "tendency" of the ions to achieve the stable La^{+3}, Gd^{+3} or Lu^{+3} structures.

The two chapters (13 and 14) which summarize the properties of the metals and compounds are thorough and reasonably critical. In discussing the sesquioxides, however, Vickery repeats the error found in Wells' "Structural and Inorganic Chemistry" in inverting the temperature stability of the hexagonal and cubic forms, and there is no crystallographic justification for writing a compound such as LaOCl as $La_2O_3 \cdot LaCl_3$.

Analytical methods are covered quite completely in chapter 15. Primary emphasis is on spectral methods. Irradiation activation analysis is mentioned briefly.

In the final chapter a number of uses and applications of