basis of its different color response in the latter test and its different rate of migration in 88% phenol and in butanol–pyridine–water (10:3:3).[10]

The urine of normal guinea pigs and of three normal humans was free of xylulose. The urine of a fourth subject yielded the pentose, as indicated by spectrophotometric examination of the colors obtained in the orcinol[5] and cysteine-carbazole tests, as well as by paper chromatographic comparisons in several different solvents. The pentose was excreted by all four subjects after the ingestion of 17 g. of D-glucuronolactone per day and by guinea pigs fed 0.1 g. of D-glucuronolactone per day. The amounts excreted were approximately 25 to 50 mg. and 1 to 5 mg., respectively. To demonstrate conclusively that the pentose being studied was actually L-xylulose, L-xylosazone (m.p. 161–162.5°) was prepared from a chromatographically purified fraction of the urine of the subject who normally excreted the sugar, glucuronolactone having been fed to increase the amount of pentose excreted. Recrystallization of this product, after it was mixed with an equal amount of pure D-xylosazone, yielded the characteristic crystals of DL-xylosazone (m.p. 198–198.5°).[4]

In contrast to normal humans, pentosurics usually excrete 1.5 to 2.0 g. of L-xylulose daily and twice this amount after ingesting only 5 g. of D-glucuronolactone.[11] It is possible that the pentose is a normal metabolite which is utilized poorly by pentosurics. We are endeavoring to obtain information bearing on this hypothesis. No L-xylulose could be found in the urine of a mouse which had been given intraperitoneally 50 mg. of the pentose (equivalent to 100 g. in a human being).[12] The guinea pig liver experiments were based on similar studies on D-fructose.[13] The liver slices utilized L-xylulose more rapidly than D-ribose or any other pentose tested, and the rate was equal to that of fructose. Homogenates also caused rapid disappearance of the pentose. Unlike fructose, however, the utilization was inhibited by fluoride, and it did not require the addition of adenosine triphosphate if L-glutamate was present.

(10) L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 4047 (1952).

(11) These values are given in reference 4, and we have confirmed them by chromatographic analyses.

(12) The mouse was chosen for this experiment because of the limited quantity of L-xylulose available. It was isolated from the urine of a pentosuric individual (G. W.), to whom we are indebted for supplying us with urine and for participating in experiments involving glucuronolactone.

(13) C. S. Vestling, A. K. Mylroie, U. Irish and N. K. Grant, *J. Biol. Chem.*, **185**, 789 (1950).

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CARBOXYPEPTIDASE, A ZINC METALLOPROTEIN

Sir:

We have recently examined several crystalline preparations of pancreatic (bovine) carboxypeptidase utilizing quantitative emission spectrography. Analyses were performed in duplicate with a porous cup spark excitation system. A Jarrel-Ash varisource in conjunction with a 21-foot Wadsworth spectrograph having a plate factor of 5.18 Å per mm. in the first order was employed.[1] Qualitative and quantitative spectrographic analyses of several crystalline preparations uniformly indicated the presence of large quantities of zinc, while all other metals were either completely absent or present in minute quantities. Table I gives typical quantitative data on a six times recrystallized preparation, prepared by one of us (H. N.),[2] having maximum enzymatic activity toward the substrate carbobenzoxyglycyl-L-phenylalanine[3] and free of proteolytic activity toward substrates of other enzymes (chymotrypsin and trypsin); 1820 micrograms of zinc per g. dry weight of protein were found spectrographically. Zinc measurements were independently confirmed in this and all other analyses (see Table I) by means of a microchemical technique previously described[4] utilizing diphenylthiocarbazone as a colorimetric agent. The molecular weight of carboxypeptidase has been determined[5,6] to be 34,300 and the mole ratio of zinc/carboxypeptidase, for this preparation, is, therefore, 0.96, indicating 1 mole of zinc per mole of carboxypeptidase. Other preparations, from our own and commercial sources, have given similar zinc and other metal distributions and molar ratios.

It will be noted that the amounts of barium, used in the extraction of the enzyme, are minimal in the crystalline preparation. Lithium, employed in recrystallization, could not be detected. All other alkali metals and alkaline earths, including beryllium, magnesium and calcium, were either completely absent or did not exceed a maximum concentration of 5.8 micrograms per gram of protein. In one preparation, no magnesium could be detected at all, either by this or a flame spectrophotometric method.

TABLE I

EMISSION SPECTROGRAPHIC ANALYSIS OF SIX TIMES RECRYSTALLIZED CARBOXYPEPTIDASE

Element	Line / internal standard	μg per g. of carboxypeptidase
Zinc	Zn 3345/V 4111	1820.00
Zinc	By diphenylthiocarbazone	1820.00
Copper	Cu 3247/Bi 3036	33.0
Iron	Fe 3020/V 3185	39.5
Aluminum	Al 3961/V 3185	5.80
Magnesium	Mg 2779/Bi 2897	5.70
Calcium	Ca 4318/Bi 2897	2.65
Barium	Ba 4554/V 3185	18.4

Not found: Beryllium, boron, cadmium, chromium, cobalt, lead, lithium, manganese, molybdenum, nickel, phosphorus, potassium, silver, strontium, tin.

Quantitative analyses for zinc and specific activity during an entire fractionation process have shown a proportionate enrichment of both in the very fractions in which carboxypeptidase is known and was shown to be concentrated. Significantly,

(1) B. L. Vallee, in preparation for publication.

(2) *Cf.* H. Neurath, E. Elkins and S. Kaufman, *J. Biol. Chem.*, **170**, 221 (1947).

(3) E. Elkins-Kaufman and H. Neurath, *ibid.*, **175**, 893 (1948).

(4) B. L. Vallee and J. G. Gibson, 2nd, *ibid.*, **176**, 435 (1948).

(5) F. W. Putnam and H. Neurath, *ibid.*, **166**, 603 (1946); E. L. Smith, D. M. Brown and H. T. Hanson, *ibid.*, **180**, 33 (1949).

(6) E. L. Smith and A. Stockell, *ibid.*, **207**, 501 (1954).

substantial enrichment of both zinc and activity was encountered in the first crystalline fraction. Throughout the fractionation alkaline earth and transition elements decreased to absolutely, and stoichiometrically, negligible quantities.

Inhibition of enzymatic activity by typical metal chelating agents such as O-phenanthroline was observed, and, correlating to this, the agent was capable of removing zinc from the protein, which could not be accomplished by dialysis against water or ammonia. The protein satisfies the criteria of a zinc metalloprotein.[7]

These data expand on previous observations and conjectures concerning the enzymatic characteristics of carboxypeptidase and its possible metal content.[8] A full account of this work will be published elsewhere.

These studies were aided in part by a contract between the Office of Naval Research, Department of the Navy, and Harvard University, Contract No. Nr5ori-07660.

(7) B. L. Vallee, F. L. Hoch and W. L. Hughes, *Archiv. Biochem. Biophys.*, **48**, 347 (1954).

(8) H. Neurath and G. de Maria, *J. Biol. Chem.*, **186**, 653 (1950).

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RECEIVED AUGUST 11, 1954

BOOK REVIEWS

Catalysis. Volume I. Edited by PAUL H. EMMETT, Gulf Research and Development Company's Multiple Fellowship, Mellon Institute, Pittsburgh, Pennsylvania. Contributing Authors are HERMAN E. RIES, JR., PAUL H. EMMETT, KEITH J. LAIDLER, W. B. INNES, F. G. CIAPETTA C. J. PLANK, and P. W. SELWOOD. Reinhold Publishing Corporation, 330 W. 42nd Street, New York 36, N. Y. 1954. vi + 394 pp. 16 × 23.5 cm. $10.00.

During World War I, Rideal and the writer wrote a text entitled "Catalysis in Theory and Practice." There was a good deal of descriptive chemical technology and very little theory or science. That has all changed and the subject of catalysis now requires two volumes "to cover all the general approaches and tools" necessary to obtain a basic understanding of catalysis. An unspecified number of subsequent volumes will cover principles and mechanisms in individual catalytic reactions. The Editor selected for the task is a guarantee of a careful, thorough and competent coverage of the vast field. The present Volume I is at once a confirmation of this view and builds up one's expectancy for the volumes to follow.

In the order of the contributing authors listed above the topics covered in Volume I are Physical Adsorption; Surface Area; Chemisorption, Kinetic Laws, and Absolute Rates (3 chapters by Laidler); Catalyst Carriers, Promoters, Accelerators, Poisons and Inhibitors; Catalyst Preparation; Magnetism and Catalysis.

It is a striking feature of this field of work that new developments succeed each other with such speed that chapters in a book need additions even before publication. This is exemplified in the chapter on physical adsorption where new work by W. R. Smith, Beebe, Halsey and others in the last year has further illuminated the factors operative in the structure of the adsorption isotherms, and the role of the surface and its uniformity in the process. The data on chemisorption are excellently presented but again, in this case, newer data are beginning to suggest variations from the point of view presented. It is certain that the subject is actually more complex than the presentation of Laidler would suggest. The induced heterogeneity of even a uniform surface which is produced by chemisorption indicates that in calculating the absolute rates of surface reactions, the concept of concentration of sites cannot be the only parameter. The technical excellence of the book is admirably exemplified in Innes' chapter on carriers, promoters, etc. Throughout the book there is a wealth of tabular information. The chapter on catalyst preparation gives comprehensive literature references as well as important experimental examples. The final chapter on magnetism by Selwood gives an authoritative outline of theory and well-chosen examples of applications of magnetism to the identification of catalyst components, reactions in the solid state with appropriate exemplification of experimental methods.

Volume I of the new text on catalysis is a heartening record of the progress that has been achieved in the science of catalysis during the last three decades and an indispensable base from which to proceed to chart the unknown future in the field.

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Advances in Protein Chemistry. Vol. VIII. Edited by M. L. ANSON, Lever Brothers Company Research Center, Edgewater, New Jersey, KENNETH BAILEY, University of Cambridge, Cambridge, England, and JOHN T. EDSALL, Harvard Medical School, Boston, Massachusetts. Academic Press, Inc., Publishers, 125 East 23rd Street, New York 10, N. Y. 1953. ix + 529 pp. 16 × 23.5 cm. Price, $10.50.

The eighth volume of "Advances in Protein Chemistry" maintains the high standards set by the preceding volumes in this series. It will undoubtedly find its way to every protein chemist's bookshelf. As in previous years, the reviews, of which there are seven, are authoritative, comprehensive and unhurried. One has the feeling that the authors have not been required to limit themselves to any given number of pages.

As usual, the interests of all varieties of protein chemists are represented. The articles range from "Rotational Brownian Motion and Polarization of the Fluorescence of Solutions" by G. Weber, a review distinguished by detailed mathematical derivation of pertinent equations, to "Peanut Protein, Isolation, Composition and Properties" by J. C. Arthur, Jr., a review emphasizing earthy facts, such as U. S. production figures for peanut butter (about 5 lb. per person per year, a figure well below the rate of consumption by the younger members of this reviewer's household).

The other articles are: "Naturally Occurring Peptides" by E. Bricas and C. Fromageot (with 667 references); "Peptide Bond Formation" by H. Borsook; "Bacteriophages: Nature and Reproduction" by F. W. Putnam; "Assimilation of Amino Acids by Gram-Positive Bacteria and Some