sides in a discrete band, the center of which varies from R_f 0.33 to 0.40. Another band at approximately R_f 0.57 corresponds to the free base and the sulfadiazine is at 0.86. The 0.40 fraction is eluted with water and after lyophilizing rechromatographed on 2 sheets of filter paper with 60% *n*-propanol in water in the presence of a beaker of 1 *M* NH_4OH. The compound shows an R_f of approximately 0.65. A third chromatographing of the compound for 24 hr. at room temperature with the organic phase of a mixture of *n*-butanol, acetic acid and water in the volume proportions of 4:1:5 allowing the solvent to run off the paper removes carbohydrate impurities. Assigning a migration value of 1.0 to D-ribose, the pentoside moves to 1.19. The resulting compound remains as a single orcinol-reacting, diazotizable, non-acetylatable amine through chromatography with a wide variety of solvents. In seven solvents the movement of the pentoside on paper is slower than that of the carboxamide. The movement of the compound on paper and its analysis precludes its containing a phosphate ester.

This carboxamide compound shows a maximum absorption at about 267 mμ at *p*H 7 with a curve closely similar to that of the free base. Analysis of the imidazolecarboxamide and pentose moieties of the riboside in terms of ultraviolet absorption maximum, diazotizable amine[9] and orcinol reaction shows these relative molar ratios

Density 267 mμ[a]	Diazotizable amine[a]	Pentose[b]
1.17	1.00	1.00

[a] Standard: 4-NH_4-5-imidazolecarboxamide based on $\epsilon_{267\ m\mu}$ of 1.27×10^4. [b] Method of W. Mejbaum,[10] heating 40 min.; standard, recrystallized D-ribose.[11]

The variation from 1:1:1 may be ascribed in part to the probable difference between the ultraviolet extinction coefficients of the free base and the riboside.

Hydrolysis of carboxamide riboside with 0.5 *N* hydrochloric acid 30 min. at 100° liberates the free carboxamide as demonstrated by chromatographing the hydrolysate with four solvents and comparing the R_f values, ultraviolet absorption and diazotization reaction of the base which is formed with that of the authentic imidazolecarboxamide. Acid hydrolysis liberates an aldose-reacting sugar which tentatively may be considered to be ribose. Using paper chromatography with four solvents, the sugar corresponds to D-ribose rather than arabinose, lyxose, or xylose and shows a pink color with aniline phthalate reagent.[12] The absorption spectrum of the Bial orcinol reaction product of the riboside corresponds closely to that of the aldopentoses. The nature or position of the riboside linkage is not certainly defined. Since dilute acid effects hydrolysis of the glycosidic linkage and since the ultraviolet absorption spectrum of the riboside is similar to that of the free base, it is assumed that the ribose is attached to the nitrogen which would correspond to the number 9 position in the purine.[13] 4-Amino-5-imidazolecarboxamide riboside is unstable on standing on paper, being converted to a red compound. Thus the yield tends to be low (approximately 30%) but is dependent on the care taken. It is apparent that the earlier method of isolating the carboxamide[1,2] may have caused acid hydrolysis of its ribose derivatives.

Recently Ben-Ishai and co-workers[14] tentatively identified the desoxyriboside of carboxamide as the product of the incubation of the carboxamide with *E. coli* suspensions, and MacNutt[15] employing *Lactobacillus helveticus* extracts has presented evidence for transglycosidation from thymidine to imidazolecarboxamide to form the carboxamide desoxyriboside. In the present studies the riboside is synthesized by a *de novo* pathway as is shown by the fact that glycine-1-C^{14} is converted to the carboxamide moiety of the riboside with little dilution.

The demonstration of the occurrence of this compound as the major component of the diazotizable compounds in these young cultures does not necessarily constitute evidence that *per se* it is an intermediate reactant in purine nucleotide synthesis. It may in fact represent a degradation product perhaps from the ribotide. Details of experiments regarding the formation of the carboxamide compounds and the large scale isolation of the riboside will be presented elsewhere.

DEPARTMENT OF BIOCHEMISTRY
WESTERN RESERVE UNIVERSITY
SCHOOL OF MEDICINE G. ROBERT GREENBERG[16,17]
CLEVELAND 6, OHIO

RECEIVED NOVEMBER 28, 1952

(9) W. Mejbaum, *Z. physiol. Chem.*, **258**, 117 (1939).

(10) W. Mejbaum, *Z. physiol. Chem.*, **258**, 117 (1939).

(11) L. Berger, U. V. Solmssen, F. Leonard, E. Wenis and J. Lee, *J. Org. Chem.*, **11**, 91 (1946).

(12) S. M. Partridge, *Nature*, **164**, 443 (1949).

(13) J. M. Gulland and E. R. Holiday, *J. Chem. Soc.*, 765 (1936).

(14) R. Ben-Ishai, E. D. Bergmann and B. E. Volcani, *Nature*, **168**, 1124 (1951).

(15) W. S. MacNutt, *Biochem. J.*, **50**, 384 (1952).

(16) Aided by grants from The National Foundation for Infantile Paralysis, and the Elisabeth Severance Prentiss Foundation.

(17) I wish to thank Mrs. Hale Bumpus for her valuable assistance.

THE PHOSPHORUS IN PEPSIN AND PEPSINOGEN

Sir:

If the molecular weights of pepsin and pepsinogen are 35,000 and 38,000, respectively,[1] each of these molecules contains one atom of phosphorus.[2,3] Since it has been shown that ovalbumin and α-casein are dephosphorylated by certain phosphatases from mammalian tissue, and from potato,[4,5,6] it was of interest to study the action of these enzymes on pepsin and its precursor. If dephosphorylation occurs, the influence of this reaction on the proteolytic activity of pepsin and on the pepsinogen-pepsin transformation becomes of considerable interest.

The pepsinogen used in this work was kindly supplied by Dr. Roger M. Herriott of the School of Hygiene and Public Health of Johns Hopkins University, while the crystalline pepsin was a Worthing-

(1) Northrop, Kunitz and Herriott in "Crystalline Enzymes" Columbia University Press, New York, 2nd edition, 1948, p. 74 and 81.

(2) Northrop, *J. Gen. Physiol.*, **13**, 739 (1930).

(3) Herriott, *ibid.*, **21**, 501 (1938).

(4) Perlmann, *ibid.*, **35**, 711 (1952).

(5) Perlmann, THIS JOURNAL, **74**, 3191 (1952).

(6) Perlmann in "Phosphorus Metabolism, 11," Johns Hopkins University Press, Baltimore, Md., 1952, in press.

ton product. To a 20-mg. sample of protein in 2.2 ml. of 0.1 molar sodium acetate buffer of *p*H 5.6 was added 66 μg. of potato phosphatase[7] and the mixture incubated at 37°. In order to determine proteolytic activity as well as the liberated inorganic phosphorus, the reaction mixture was divided, after incubation, into two parts. To a 1-ml. portion, an equal amount of 20% trichloroacetic acid was added, the mixture immersed in boiling water for 10 minutes and the inorganic phosphorus determined after centrifugation of the precipitated protein. With the aid of the hemoglobin method[8] the second sample was assayed for proteolytic activity. The results, together with additional experimental details, are summarized in Table I.

As shown in Table I, potato phosphatase dephosphorylates pepsin and pepsinogen without loss of its proteolytic activity. This indicates that the phosphorus in these proteins is not essential for either the enzymatic activity of pepsin or the pepsinogen–pepsin transformation.

Although not shown in Table I, intestinal phosphatase also dephosphorylates pepsin but at *p*H 8.9. Exposure of the protein to this alkaline reaction results in loss of the proteolytic activity. Pepsinogen is also dephosphorylated by intestinal phosphatase. The latter protein, however, is stable at *p*H 8.9 and proteolysis of hemoglobin is observed on subsequent conversion to pepsin at *p*H 2. In contrast with the potato and intestinal phosphatase, that derived from prostate and active at *p*H 5.3 does not act on pepsin. Since this is the enzyme that removes selectively the phosphate group that is lost in the $A_1 \rightarrow A_2$ transformation of ovalbumin,[4] the linkage of the phosphorus in pepsin is probably different from that of the A_1 component of ovalbumin that is attacked by the prostate enzyme.

(7) Koruberg, unpublished.

(8) Anson in Northrop, Kunitz and Herriott, "Crystalline Enzymes," Columbia University Press, New York, N. Y., 2nd edition, 1948, p. 305.

TABLE I

ACTION OF POTATO PHOSPHATASE ON PEPSIN AND PEPSINOGEN

Reaction mixture	Time of incubation at 37° in hours	Phosphorus released per 1 ml. protein soln., μg.	Phosphorus released by enzyme % of total phosphorus	Relative specific proteolytic activity[a]
Pepsin	0	0	0	100
Pepsin	24	0	0	96
Pepsin + potato phosphatase	24	8.24	99.0	96
Pepsinogen	0	0	0	100
Pepsinogen	24	0	0	98
Pepsinogen + potato phosphatase	24	7.7	96.0	113

[a] The relative specific activity of a freshly prepared enzyme solution is taken as 100.

I wish to express my sincere thanks to Dr. B. L. Horecker of the National Institutes of Health, Bethesda, and to Dr. G. Schmidt of the Boston Dispensary, Boston, for the samples of phosphatases used in this work.

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, N. Y.
GERTRUDE E. PERLMANN

RECEIVED NOVEMBER 5, 1952

BOOK REVIEWS

Polarography. Second Edition. Volume I. **Theoretical Principles, Instrumentation and Technique.** By I. M. KOLTHOFF, Professor and Head of Division of Analytical Chemistry, University of Minnesota, Minneapolis, Minnesota, and JAMES J. LINGANE, Professor of Chemistry, Harvard University, Cambridge, Massachusetts. Interscience Publishers, Inc., 250 Fifth Avenue, New York 1, N. Y. 1952. xvii + 420 pp. 16 × 23.5 cm. Price, $9.00.

All those interested in polarography will welcome the publication of the second edition of this authoritative treatise. As the authors point out in the preface of this new edition, the polarographic literature has tripled in size since the publication of the first edition in 1941, and the book, in its new edition, is divided into two volumes covering the fundamentals and the applications, respectively. The present review covers the first volume.

The material has been brought up to date by extensive revision of the text and by the addition of several new chapters. As a result, the text of the first volume is almost twice as long as its equivalent in the first edition.

Part I covers the theoretical principles of polarography. The basic principles of polarography and voltammetry are discussed in Chapter I. The theory of the diffusion current is extensively discussed in the following three chapters. The reader is guided very firmly in mathematical derivations in these and other chapters. Derivations are thoroughly presented and the various steps in the mathematical reasoning are clearly indicated. Recent developments such as modified forms of the Ilkovic equation, experimental studies of the influence of the capillary characteristics on the diffusion current, and current–time curves are reviewed. Following a chapter on polarography in non-aqueous media, there is a detailed discussion of the various factors affecting the diffusion current. This thorough exposé of the theory of the diffusion current is followed by six chapters dealing with polarographic waves. The very difficult problems presented by polarographic maxima are discussed in Chapter X; analytical applications of the suppression of maxima are reviewed in the same chapter. The theory of reversible waves was well developed at the time of the publication of the first edition, and virtually no change was made in this part of the book, except for the introduction of a new section on dropping amalgam electrodes. In contrast, much new material is presented in Chapters XIV and XV, in which the remarkable studies of the Czechoslovak school on kinetic, catalytic and adsorption waves are reviewed. The Brdicka–Wiesner theoretical treatment based on the concept of the thickness of reaction layer is used throughout the discussion, and the reader is duly warned about the approximate nature of the derivations. The more rigorous