obtained from many experiments, is 41% in the first, 31% in the second, and 28% in the third peak. Assay of each of these fractions by the procedure of Sayers, *et al.*,[8] reveals the ACTH activity to be concentrated almost completely in the second peak. Thus a 2- to 4-fold purification is achieved (potency approximately 160 times standard). An oxycellulose-purified pig ACTH preparation,[9] when submitted to this type of analysis, revealed a distribution of 33, 54 and 13% in the first, second and third peaks, respectively.

The evidence for non-equilibrium in the system described is deduced mainly from the fact that the substances present in the first two peaks can be displaced almost completely to the last peak by means of flow rates which are much slower than those reported above.

Purification of Preparation E was followed by filter paper electrophoresis[10] and by two-dimensional paper chromatography of complete hydrolysates of the material in each of the peaks.[11] At *p*H 8, electrophoresis demonstrated that the first peak contained all of the anionic component(s), and the last peak the strongly cationic component(s). The middle peak showed a distribution of material at the origin or near it on the cathode side, areas where the activity has been found previously under these conditions.[4] Amino acid analyses reveal the first peak to contain all the amino acids found in the original preparations. The second peak contains only lysine, aspartic and glutamic acids (probably as amides), glycine, alanine, valine, leucine, phenylalanine, proline, and arginine (?). The third peak contains a large amount of arginine in addition to the amino acids found in the middle peak. Because of the high ultraviolet absorption of all fractions, tryptophane is presumed to be present in all three.

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(8) M. A. Sayers, G. Sayers and L. A. Woodbury, *Endocrinology*, **42**, 379 (1948).

(9) Supplied through the kindness of Dr. E. B. Astwood.

(10) H. G. Kunkel and A. Tiselius, *J. Gen. Physiol.*, **35**, 118 (1951).

(11) We are indebted to Dr. A. L. Levy for the amino acid analyses.

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THE EFFECTS OF DIVALENT CATIONS ON THE ENZYMATIC ACTIVITIES OF TRYPSIN AND OF α-CHYMOTRYPSIN[1]

Sir:

In view of current efforts to elucidate the mode of action of trypsin and α-chymotrypsin on synthetic substrates and inhibitors,[2] we wish to call attention to the fact that certain divalent cations which stabilize trypsin[3] also increase the enzymatic activity of trypsin and α-chymotrypsin.

The trypsin-catalyzed hydrolysis of 0.005 and 0.01 *M* benzoyl-L-arginine ethyl ester[4] at *p*H 7.8 and 25° is appreciably accelerated by some divalent cations, unaffected by others, whereas others again cause substantial inhibition. Twice recrystallized trypsin (Worthington, lot T-340), was dialyzed against 0.001 *M* HCl until salt-free, and lyophilized. The effects of divalent cations, added either as chlorides or sulfates, were tested in the presence of 0.005 *M* tris-(hydroxymethyl)-aminomethane·HCl buffer (THAM). Zero order reaction kinetics was observed in every case.

It is evident from the table that Ca^{++}, Mn^{++}, Cd^{++} and Co^{++} are most effective in increasing the activity of trypsin (by about 30%), Mg^{++} and Ba^{++} being somewhat less effective. The activation by Ca^{++} or Co^{++} is completely reversed by ethylenediamine tetraacetate (Versene). Trypsin is inhibited, in order of increasing effectiveness, by Zn^{++}, Hg^{++} and Cu^{++}. After partial inhibition by Cu^{++} (in borate buffer) partial reactivation by Ca^{++} can be brought about. The activity of Cu^{++}-inhibited trypsin (0.0005 *M* Cu^{++}) can be restored by Versene (0.001 *M*) and further activation is brought about by the subsequent addition of Ca^{++} (0.01 *M*).

TABLE I

EFFECT OF DIVALENT CATIONS ON THE HYDROLYSIS OF BAEE BY TRYPSIN

Cation added	Relative activity[a] 0.001 *M*	0.01 *M*
None	1.00	
Ca^{++}	1.28	1.28
Mn^{++}	1.34	1.35[b]
Cd^{++}	1.34	
Co^{++}	1.18	1.30[b]
Mg^{++}	1.09	1.26
Ba^{++}	1.08	1.28
Sr^{++} or Ni^{++}		1.10
Fe^{++} or Pb^{++}	About 1.0	
Zn^{++}	0.81	
Cu^{++} (borate buffer)	0.0	
Hg^{++}	0.0	
Versene, 0.01 *M*	1.00	
Co^{++} + Versene (0.012 *M*)	1.00	
Ca^{++} + Versene (0.012 *M*)	1.00	

[a] Activity expressed as ratios of zero order reaction constants in the presence of the added cation to that observed in 0.005 *M* tris-(hydroxymethyl)-aminomethane·HCl buffer, *p*H 7.8, alone. [b] Corrected for effect of partial precipitation of the metal oxide on alkali consumption during ester hydrolysis.

When these divalent cations were tested for their effect on the α-chymotrypsin catalyzed hydrolysis of 0.01 *M* acetyl-L-tyrosine ethyl ester[5] in 0.005 *M* THAM buffer, *p*H 7.8, only Ca^{++} produced significant activation. Maximum activation of about 50% was attained in the presence of 10^{-2} *M* Ca^{++} and one-half of that value was attained in 2×10^{-4} *M* Ca^{++}. In 0.001 *M* solutions, Zn^{++} yielded 50% inhibition, and Cu^{++} and Hg^{++} complete inhibition. α-Chymotrypsin completely inhibited by 10^{-3} *M* Cu^{++} could be partially reactivated by

(1) This work was supported by grants from the Public Health Service, the Rockefeller Foundation and the Lilly Research Laboratories, Eli Lilly and Company.

(2) H. Neurath and G. W. Schwert, *Chem. Rev.*, **46**, 69 (1950); H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 1541, 1555, 3223, 3231 (1951); *ibid.*, **74**, 101 (1952).

(3) M. Bier and F. F. Nord, *Archiv. Biochem. Biophys.*, **33**, 320 (1951); L. Gorini, *Biochim. Biophys. Acta*, **7**, 318 (1951).

(4) G. W. Schwert and M. A. Eisenberg, *J. Biol. Chem.*, **179**, 665 (1949).

(5) S. Kaufman and H. Neurath, *Arch. Biochem.*, **21**, 437 (1949); G. W. Schwert and S. Kaufman, *J. Biol. Chem.*, **180**, 517 (1949).

Ca^{++} or Versene. The present enzyme preparations contained only traces of Ca^{++}, as measured by flame spectrophotometry (0.02% in trypsin and 0.004% in chymotrypsin).

The activation effects described herein, which were also observed with the corresponding amide substrates, are of smaller magnitude and therefore probably of a different type than those usually associated with metal activation of enzymes.[6] The suggestion that the binding of these cations by the protein involves a shift of the equilibrium among coexistent forms of varying enzymatic activity[3] deserves serious consideration.

Further quantitative studies on trypsin, chymotrypsin and carboxypeptidase are now in progress, and together with the details of the present report, will be published at a later date.

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(6) A. L. Lehninger, *Physiol. Rev.*, **30**, 393 (1950).
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THE ENZYMATIC FORMATION OF SEDOHEPTULOSE PHOSPHATE FROM PENTOSE PHOSPHATE

Sir:

Sedoheptulose (D-altroheptulose), originally discovered in the Sedum plant,[1] has recently been reported to occur as a phosphate ester among the early products of photosynthesis.[2] The hexosemonophosphate fraction isolated from yeast also has been found to contain about 2% of a heptulose ester.[3] We have now identified sedoheptulose phosphate as a product of pentose phosphate metabolism with purified enzymes of animal origin.

Enzymes in red cells[4] and in bacteria and yeast[5] which split pentose phosphate to form triose phosphate have been described. We have purified a similar enzyme about 60-fold from rat liver acetone powder extracts by fractionation with ammonium sulfate, methanol and acetone. The enzyme assay was based on the rate of oxidation of reduced diphosphopyridine nucleotide in the presence of α-glycerophosphate dehydrogenase and triose phosphate isomerase.[6] The purified preparation contains pentose phosphate isomerase but has greater activity with ribulose-5-phosphate than with ribose-5-phosphate. Neither of these substrates is attacked appreciably by the purified liver enzyme unless a system for the removal of the products is added. In the presence of the purified pentose-splitting enzyme and crystalline muscle aldolase,[7] there is virtually complete removal of pentose phosphate and a recombination of the fragments to form sedoheptulose phosphate. During this process the absorption band at 670 mμ due to pentose in the orcinol reaction is replaced by a band at about 600 mμ, which is identical with that obtained with sedoheptulose. From 2 moles of pentose phosphate approximately 1 mole of sedoheptulose phosphate and about 0.5 mole of triose phosphate were formed (Table I). No explanation is available for the low yield of triose phosphate. The identification of the triose as dihydroxyacetone was based on paper chromatography with acetone–water mixtures. Sedoheptulose was converted to sedoheptulosan tetrabenzoate[8] after hydrolysis of the reaction mixture with a purified potato phosphatase.[9] The derivative melted at 164.5–165°, as did an authentic sample,[10] and the mixed melting point was 163.5–164°. The optical rotation was $\alpha^{20}D$ −194° (c = 0.72 in $CHCl_3$) compared to $\alpha^{20}D$ −195° for the authentic derivative.

TABLE I

STOICHIOMETRY OF PENTOSE PHOSPHATE CONVERSION[a]

	Micromoles		
	0 min.	60 min.	Δ
Pentose phosphate	6.26	1.48	−4.78
Sedoheptulose phosphate[b]	0	2.43	+2.43
Triose phosphate[c]	0	1.14	+1.14

[a] The reaction mixture contained 0.36 mg. of purified pentose-splitting enzyme and 0.19 mg. of recrystallized muscle aldolase in 1.1 cc. of 0.01 *M* glycylglycine buffer *p*H 7.4 containing 0.01 *M* cysteine. Incubation was at 23°. [b] Calculated from the absorption at 580 mμ in the orcinol pentose method of W. Mejbaum, Z. *physiol. Chem.*, **258**, 117 (1939). [c] Determined by oxidation of reduced diphosphopyridine nucleotide in the presence of α-glycerophosphate dehydrogenase. Since the latter preparation contains aldolase and triose phosphate isomerase, the determination measures fructose diphosphate and glyceraldehyde-3-phosphate, as well as dihydroxyacetone phosphate.

Heptulose phosphate is also formed on incubation of D-erythrose with hexosediphosphate and aldolase. This observation suggests that sedoheptulose phosphate formation from pentose phosphate proceeds by way of a tetrose, derived from 2 two-carbon fragments from pentose phosphate, which under the influence of aldolase condenses with dihydroxyacetone phosphate to yield sedoheptulose phosphate.

(1) F. B. LaForge and C. S. Hudson, *J. Biol. Chem.*, **30**, 61 (1917).
(2) A. A. Benson, J. A. Bassham and M. Calvin, THIS JOURNAL, **73**, 2970 (1951).
(3) R. Robison, M. S. Macfarlane and A. Tazelaar, *Nature*, **142** 114 (1938).
(4) Z. Dische, *Naturwiss.*, **26**, 252 (1938).
(5) E. Racker in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The John Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 147.
(6) E. Racker, *J. Biol. Chem.*, **167**, 843 (1947).
(7) J. F. Taylor, A. A. Green and G. T. Cori, *ibid.*, **173**, 591 (1948).
(8) W. T. Haskins, R. M. Hann and C. S. Hudson, THIS JOURNAL, in press.
(9) A. Kornberg, unpublished procedure.
(10) Prepared from sedoheptulosan generously supplied by Dr. N. K. Richtmyer.

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SURFACE CHEMICAL PROPERTIES OF SOLIDS COATED WITH A MONOLAYER OF PERFLUORODECANOIC ACID[1]

Sir:

Attention has been directed recently to the modification of the surface chemical properties of a solid

(1) Taken from a thesis submitted by Fred Schulman in partial fulfillment of the requirements for the degree of Ph.D. at The Division of Chemistry, Graduate School, Georgetown University, Washington, D. C.