Fractionation of Amino Acid Mixtures in Acetone by Means of Alkyl Acid Phosphates.* (17551)

E. V. McCollum, Agatha A. Rider and Hanna Suss

From The Johns Hopkins University, Baltimore, Md.

In a former communication(1) we described the property of solvating amino acids in dry acetone or dioxane, which is possessed by 8 organic acids, di- and trichloroacetic acids, and certain sulfonic acids. The purpose of the present communication is to state that we have found that several alkyl acid phosphates† also possess the property of solvating a dry mixture of amino acids in dry acetone. These may be used for dividing a protein hydrolysate into a series of fractions containing one to six or seven amino acids, certain of these composing a high percentage of the individual fractions. On adding ammonia to acetone solutions of amino acids formed by the aid of alkyl acid phosphates, both amino acids and the ammonium salts of the reagent-acids precipitate together. This property of alkyl acid phosphates contrasts strongly with that of many sulfonic acids, which, under these conditions, form salts with ammonia which are readily soluble in acetone, permitting the precipitated amino acids to be separated from the solvating agent. The triethylamine (T.E.A.) salts of the alkyl acid phosphates are soluble in acetone. This property permits the amino acids to be separated from the reagent by means of this base.

Individual amino acids show marked differences in solubility in an acetone solution of octyl, n-amyl, iso-amyl, n-butyl, iso-butyl, n-propyl and iso-propyl acid phosphates. Table I illustrates the contrasting solvating power of 5 reagent acids for 9 amino acids. We designate by the term proline equivalent, the ratio of the amount of a reagent necessary to dissolve one equivalent of amino acid to the amount of the same reagent necessary to dissolve one equivalent of proline.

The amino acids in a hydrolysate exert marked influence on each other's solubility in acetone-alkyl acid phosphate solutions. Much less acetone-reagent is required to dissolve a given weight of dry protein hydrolysate than is necessary to dissolve the component amino acids of the sample in the isolated state. There is, however, pronounced selectivity in the order in which certain amino acids are dissolved when successive portions of an acetone-reagent are passed through a sample of hydrolysate contained in a filtering crucible.

Table II illustrates some contrasting properties of alkyl acid phosphates in solvating power for a casein hydrolysate.‡ It also shows the differences in extent of precipitation of amino acids from their acetone-reagent-hydrolysate systems after neutralization with triethylamine, and the degree of complexity of the more difficultly soluble residues not brought into solution in our experiments.

A Procedure for Fractionation of a Protein Hydrolysate. A 10 g sample of the dry, finely ground, hydrolysate is thoroughly mixed with an equal weight of anhydrous calcium sulfate (Drierite), and the mixture is placed in a sintered glass crucible equipped with filter flask and suction connection. A test tube is placed inside the filter flask to collect the filtrate. The amino acid-Drierite mixture is thoroughly wet with the reagent (*e.g.* a 0.2 N solution of an alkyl acid phosphate in acetone), the contents of the crucible are stirred for a definite time (usually one minute), suction is applied, and the solution in the crucible is sucked through into the test tube. Another portion of the reagent is then added, and the

* This investigation was supported in part by a grant-in-aid from Nutrition Foundation, Inc.

1. McCollum, E. V., and Rider, Agatha A., *Science,* 1948, v108, 111.

† The authors are indebted to Monsanto Chemical Co., St. Louis, Mo., through the courtesy of Mr. Paul Logue, for the samples of alkyl acid phosphates employed in this investigation.

‡ The authors are indebted to National Dairy Products Corp. Research Laboratories, Oakdale, L.I., through the courtesy of Dr. Arnold H. Johnson, for the sample of hydrochloric acid casein hydrolysate, their "Hycase," a salt-free preparation.

TABLE I.
Proline Equivalents of Alkyl Acid Phosphates in 0.2 N Solutions in Acetone.

Amino acid	Octyl	n-Amyl	n-Butyl	n-Propyl	Isopropyl
Proline	1	1	1	1	1
Isoleucine	3	2	4	3	1
Ph. alanine	6	6	7	2	2
Leucine	6	4	5	13	2
HO-proline	12	7	14	5	3
Tryptophan	14	3	7	2	1
Threonine	16	5	7	4	2
Serine	42	42	19	9	11
Glutamic acid	58	70	42	23	8

TABLE II.
Solvating Power of 0.2 N Solutions of Alkyl Acid Phosphates, Precipitates Yielded on Neutralization with Triethylamine (T.E.A.), and Chromatographs of an Undissolved Residue.

Alkyl acid phosphate	% of sample of a casein hydrolysate readily soluble‡	% of soluble material precipitated on addition of T.E.A.	Results of chromatographing the insoluble material	Remarks
Octyl	97	85	—	
n-Amyl	45	80	—	*
Iso-amyl	95	75	3 spots	
n-Butyl	97	68	—	
Iso-butyl	97	80	1 spot	Tyrosine
n-Propyl	97	90	2 spots	
Iso-propyl	100	45	—	†

* The amyl acid phosphate-insoluble material has been provisionally identified as the basic amino acids, methionine, tyrosine (and possibly serine), alanine, proline, valine, and the leucines. This insoluble fraction contains all of the tyrosine. The moiety which is soluble in amyl acid phosphate, and precipitable with triethylamine, has been tentatively identified as a mixture of some of each of the basic amino acids, aspartic acid, glutamic acid (and possibly serine), phenylalanine, and/or valine, alanine, methionine, proline, and the leucines. All of the glutamic and aspartic acids are found in the soluble and precipitable material. The soluble material which remains in solution after neutralization with T.E.A. appears to contain almost all of the proline.

† The portion of the hydrolysate which precipitates from solution with iso-propyl acid phosphate after the reagent is neutralized with T.E.A. exhibits 6 spots when examined by ascending chromatography using 77% ethanol. These spots have been shown to be arginine, histidine, lysine, glutamic acid (and possibly serine), aspartic acid, alanine, and the leucines. Glutamic acid, lysine and arginine predominate in this mixture. Histidine and aspartic acid are present only in traces.

process of stirring and filtration by suction is repeated until the desired volume of filtrate has been collected. The apparatus is then disassembled, the test tube containing the filtrate is replaced by an empty one, the apparatus reassembled, and the process of fractionation continued. The size of the filtrate fractions taken off will be determined by the number of fractions it is desired to make from the hydrolysate sample. We have made as many as 50 to 60 fractions in this manner, but experience has shown that, for our objectives, 10 to 20 fractions are all that it is worth while to make in the first fractionation. The fractions thus secured are precipitated by neutralization of the solvating acid reagent by addition of triethylamine, whereupon a certain portion of the dissolved amino acids is precipitated, as shown in Table II.

Fractionation with Amyl Acid Phosphate. When a sample of casein hydrolysate is fractionated with 0.2 N amyl acid phosphate in acetone according to the procedure described, the resulting fractions, when chromatographed, show that much simplification has occurred as compared with the original hydrolysate. The first increments of reagent which are added dissolve greater weights of hydrolysate per cubic millimeter than do later additions. These facts indicate that the solvating action of the reagent is a selective one, and that the undissolved fraction of the hydrolysate is continuously changing in composition as the fractionation proceeds.

Summary. Seven alkyl acid phosphates have been found able to solvate amino acids in dry acetone. Marked differences in solvating power are shown by these reagents for individual amino acids, and for selective solvation of amino acids from complex mixtures such as a protein hydrolysate. Mixtures of amino acids greatly influence each others' solubility when fractionation is effected by the method described.

When systems consisting of acetone-amino acid-solvating reagent, are neutralized with triethylamine, amino acids are precipitated, but never quantitatively. Marked differences occur in the extent to which the dissolved amino acids precipitate with this treatment; *e.g.* 90% were precipitated from the n-propyl, as against but 45% from the isopropyl system, when the amino acids were solvated from a dry casein hydrolysate.

These reagents offer prospects for usefulness in the fractionation of amino acid mixtures.

Received November 18, 1949. P.S.E.B.M., 1949, v72.

The Cardiac Output and Circulation Time of Ferrets.* (17552)

J. EMERSON KEMPF AND H. T. CHANG†

From the Department of Bacteriology, University of Illinois College of Medicine, Chicago.

The ferret was first used as a laboratory animal by Dunkin and Laidlaw for the study of canine distemper.(1) Subsequently it was also found to be susceptible, under experimental conditions, to other viral infections such as influenza,(2) fowlpox,(3) swineherd's disease,(4) Rift Valley fever,(5) a strain of poliomyelitis virus,(6) and lymphocytic chorio-meningitis.(7) In influenzal infection, experimental investigations employing ferrets have contributed a great deal to our knowledge of the pathology(8) and epidemiology(9) of this important disease. Other aspects of the infection, such as the problem of the toxicity of the virus and the alterations in the infected host's physiological functions are fields relatively unexplored. It seems that the ferret, because of its larger size, might serve better than the mouse or other rodents for investigations of these problems. Except for a general description by Pyle,(10) we are not aware of any detailed report dealing with the normal physiology of this animal. The present study dealing with the cardio-vascular function of normal ferrets was initiated in order to obtain such data before investigations of pathological physiology were undertaken. Cardiac output determined by the direct Fick principle, arterial blood pressure, and circulation time measurements were selected to depict the status of the animal's cardio-vascular functions.

Materials and methods. 1. *Animals*: North American ferrets (*Putorius putorius*)‡ of both sexes with body weights varying from 700 to 1100 g were used. The body surface was calculated by the formula:(11) Surface area in square meters = (body weight in g)$^{2/3}$ ×

* This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, United States Public Health Service.

† Research fellow in Medicine, University of Illinois College of Medicine.

1. Dunkin, G. M., and Laidlaw, P. P., *J. Comp. Path.*, 1926, v39, 201.

2. Smith, W., *et al.*, *Lancet*, 1933, v2, 66.

3. Findlay, G. M., and Mackenzie, R. D., *Brit. J. Exp. Path.*, 1937, v18, 146.

4. Durand, P., *C. R. Acad. Sc.*, 1936, v203, 957.

5. Francis, T., and Magill, T. P., *J. Exp. Med.*, 1935, v62, 433.

6. Patocka, F., *Zbl. Bact. Abt. I Orig.*, 1941, v148, 15.

7. Dalldorf, G., *J. Exp. Med.*, 1939, v70, 19.

8. Perrin, T. L., and Oliphant, J. W., *Public Health Rep., Wash.*, 1940, v55, 1077.

9. Commission on Acute Respiratory Diseases, *Am. J. Hygiene*, 1948, v48, 292.

10. Pyle, N. J., *Am. J. Public Health*, 1940, v30, 787.

‡ Identified by Mr. K. P. Schmidt, curator of zoology, Natural History Museum, Chicago.