



# AMINO ACID POOLS

*Distribution, Formation and Function of Free Amino Acids*

PROCEEDINGS OF A SYMPOSIUM ON FREE AMINO ACIDS  
HELD AT THE CITY OF HOPE MEDICAL CENTER,  
DUARTE, CALIF. (U.S.A.), MAY 1961

*Edited by*

JOSEPH T. HOLDEN

*Sponsored by*

*The Office of Naval Research, Washington 25, D.C.*

*Under the Auspices*

*of The Institute for Advanced Learning in the Medical Sciences*



ELSEVIER PUBLISHING COMPANY

AMSTERDAM — LONDON — NEW YORK

1962

SOLE DISTRIBUTORS FOR THE UNITED STATES AND CANADA  
AMERICAN ELSEVIER PUBLISHING COMPANY, INC.  
52 VANDERBILT AVENUE, NEW YORK 17, N.Y.

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 62-10360

WITH 1038 ILLUSTRATIONS AND 151 TABLES

ALL RIGHTS RESERVED

THIS BOOK OR ANY PART THEREOF MAY NOT BE REPRODUCED IN ANY FORM  
INCLUDING PHOTOSTATIC OR MICROFILM FORM  
WITHOUT WRITTEN PERMISSION FROM THE PUBLISHERS

PRINTED IN THE NETHERLANDS BY  
N.V. BOEKDRUKKERIJ F. E. MACDONALD, NIJMEGEN

*Organizing Committee for the Symposium:*

JOSEPH T. HOLDEN

*Chairman*

EUGENE ROBERTS

GEORGE ROUSER

*Round Table Discussions*

*edited by*

MILTON WINITZ

and

ERICH HEINZ

## PREFACE

The knowledge that living cells contain sizeable amounts of apparently uncombined, readily-extractable amino acids came to be widely appreciated at the same time that rapid chromatographic methods ideally suited to their study were developed. The prompt application of these procedures to the examination of protein-free tissue extracts has led to the accumulation of a huge volume of observations concerned with the so-called free amino acids. Despite apparent interrelations to metabolic processes, such as protein synthesis and active transport which also received intensive experimental study in these years, there has as yet been no comprehensive effort to collect, organize and evaluate this information. Some of us who have participated in various aspects of this work were acutely aware of the need for such a definitive summary and over the years have discussed numerous proposals to achieve this goal.

In discussions with representatives of the Office of Naval Research, Dr. EUGENE ROBERTS proposed the solution which is represented by this volume: a conference of leading investigators at which much of the available information would be summarized, followed by publication of the formal and informal discussions. A tentative program, developed during discussions between Dr. EUGENE ROBERTS, Dr. GEORGE ROUSER and the editor, was enthusiastically received by many of the prospective participants, and was approved for support by the Office of Naval Research. Encouraged by these favorable responses, the committee, under the editor's chairmanship, formally undertook the organization of the symposium which was held at the City of Hope Medical Center on May 19-22, 1961 under the title *Conference on Free Amino Acids*. The program consisted of three days of prepared reports interspersed with informal discussions followed by a final half-day session devoted to two spontaneous round-table discussions.

Participants were encouraged to submit extensive accounts of the work they wished to discuss in the hope that this volume would serve as a guide to a major portion of the literature on free amino acids. This goal was achieved in many, but certainly not all, cases. Authors were also encouraged to submit for publication as much original chromatographic evidence as they felt was required to document their conclusions. In the past, many journals have been reluctant to publish photographs of original chromatograms with the result that observations in this field frequently have not been adequately documented. While justified partly by the high costs involved, this editorial judgement can lead to the publication of conclusions derived from technically unsound chromatographic evidence. The publishers of this volume are to be commended for their understanding of the necessity to present this evidence in a form which permits its evaluation by the reader.

The function of the editor in this enterprise deserves comment. Participants were invited with the understanding that they would be given great latitude in the scope and method of presentation of their material. It seemed inappropriate, therefore, to embark on a vigorous program of revision in an effort to attain unity of format or a more comprehensive treatment of the subject. Only the most extreme points of view and methods of presentation were modified, generally in the interests

of accuracy and clarity of expression. Although the discussions received a less permissive treatment, an effort was made in the portions which survived editorial scrutiny to retain as much as possible of the author's mode of expression. Transcripts of the round-table discussions were edited separately by elected participants. The conferees also recommended a less specific title for this volume than was used for the conference. It was clear that some participants retain a serious doubt that the freely-extractable amino acids are uncombined within cells. To accommodate all points of view the title *Amino Acid Pools* was adopted despite some reservations of an aesthetic nature.

Many will recognize the absence of contributions from some investigators who have made pre-eminent findings in this field. In most instances, prior commitments prevented their attendance and a serious effort was made in the interests of historical accuracy and comprehensiveness of treatment to have the respective subjects discussed by their associates or collaborators.

The success of this enterprise was due in large measure to the contributions of several dedicated associates. On behalf of the organizing committee I should like to identify these as follows: Mr. DANIEL SEEDMAN, who supervised travel, housing and other physical arrangements; Mrs. HELEN WENGER, Mrs. HELEN PINKERTON and Mrs. MARGERY SIDDONS from our secretarial staff; Mr. MAX LENZ who supervised the sound equipment; and Mr. FRANKLIN A. STEINKO who produced an accurate stenotyped record of the informal discussions.

Principal financial support for the conference came from a contract between this institution and the Office of Naval Research (Contract NONR-(G)0009-61). We are indebted to Mr. LEO SHINN, Chief of the Biochemistry Branch and Dr. JOHN LOEFER of the Pasadena Branch Office for their able and sympathetic management of the requisite negotiations. The conference was held at this institution under the auspices of the Institute for Advanced Learning in the Medical Sciences which also contributed generously to its support. Additional contributions were provided by the UPJOHN COMPANY, Kalamazoo, Mich. and by the DON BAXTER COMPANY, Glendale, Calif.

JOSEPH T. HOLDEN

# CONTENTS

Introduction to Conference by E. ROBERTS (Duarte, Calif.) . . . . .	1
--	---

## PART ONE — OCCURRENCE OF FREE AMINO ACIDS

### I. Analytical Methods

Identification of the elusive amino acid by M. WINITZ (Bethesda, Md.) . . . . .	5
Discussion Chairman: E. ROBERTS . . . . .	24

### II. Plants

The soluble, nitrogenous constituents of plants by F. C. STEWARD AND J. K. POLLARD (Ithaca, N.Y.) . . . . .	25
Some recently-characterized amino acids from plants by L. FOWDEN AND D. O. GRAY (London) . . . . .	43
$\gamma$ -Glutamyl peptides in plants (Invited Discussion) by J. F. THOMPSON, C. J. MORRIS, W. N. ARNOLD AND D. H. TURNER (Ithaca, N.Y.) . . . . .	54
Free amino acids in normal and malignant plant tissues grown in tissue culture (Invited Discussion) by D. G. SIMONSEN AND E. ROBERTS (Duarte, Calif.), AND P. R. WHITE (Bar Harbor, Me.) . . . . .	65
The use of $^{14}\text{C}$ -labeled compounds in metabolic studies: effects due to autoclaving $^{14}\text{C}$ -urea (Invited Discussion) by J. K. POLLARD (Ithaca, N.Y.) . . . . .	69
Discussion Chairman: E. ROBERTS . . . . .	71

### III. Microorganisms

The composition of microbial amino acid pools by J. T. HOLDEN (Duarte, Calif.) . . . . .	73
Free amino acids in protozoa by J. B. LOEFER (Pasadena, Calif.) AND O. H. SCHERBAUM (Los Angeles, Calif.) . . . . .	109

### IV. Insects

Free amino acids in insects by P. S. CHEN (Zurich) . . . . .	115
Amino acids and derivatives in <i>Drosophila</i> by H. K. MITCHELL AND J. R. SIMMONS (Pasadena, Calif.) . . . . .	136
Metabolism of peptides in <i>Drosophila</i> by J. R. SIMMONS AND H. K. MITCHELL (Pasadena, Calif.) . . . . .	147
Discussion Chairman: N. H. HOROWITZ . . . . .	156

### V. Comparative, Developmental and Evolutionary Aspects\*

Free amino acids in invertebrates: a comparative study of their distribution and metabolism by J. AWAPARA (Houston, Texas) . . . . .	158
Free amino acids of marine invertebrates (Invited Discussion) by J. S. KITTREDGE, D. G. SIMONSEN, E. ROBERTS AND B. JELINEK (Duarte, Calif.) . . . . .	176
Lombricine and serine ethanölamine phosphodiester (Invited Discussion) by A. H. ENNOR AND H. ROSENBERG (Canberra, Australia) . . . . .	187
Discussion Chairman: N. H. HOROWITZ . . . . .	194

### VI. Vertebrates

The free amino acids of body fluids and some hereditary disorders of amino acids metabolism by R. G. WESTALL (London) . . . . .	195
--	-----

\* A paper by DR. STANLEY L. MILLER entitled: "Synthesis of amino acids in the primitive earth" was read at the conference but is not represented by an article in this volume.

Free amino acids of blood and urine in the human by P. SOUPART (Brussels) . . . . .	220
The behavior of amino acids in body fluids during development and growth: physiology and pathology by K. SCHREIER (Heidelberg, German) . . . . .	263
Free amino acids in animal tissue by E. ROBERTS AND D. G. SIMONSEN (Duarte, Calif.) . . . . .	284
Free amino acids in the blood of man and animals: I. Method of study and the effects of veni- puncture and food intake on blood free amino acids by G. ROUSER, B. JELINEK, A. J. SAMUELS, K. KINUGASA (Duarte, Calif.) . . . . .	350
Free amino acids in the blood of man and animals: II. Normal individuals and patients with chronic granulocytic leukemia and polycythemia . . . . .	373
Free amino acids in the blood of man and animals: III. Chronic lymphatic and acute leukemias by G. ROUSER, A. J. SAMUELS, D. HELLER AND B. JELINEK (Duarte, Calif.) . . . . .	388
Free amino acids in the blood of man and animals: IV. Effects of methyl (bis) $\beta$ -chloroethyl- amine (nitrogen mustard), 4-( <i>p</i> -bis(2-chloroethylaminophenyl)) butyric acid (chlor- ambucil) and phenylhydrazine by G. ROUSER, A. J. SAMUELS, K. KINUGASA, B. JELINEK AND D. HELLER (Duarte, Calif.) . . . . .	396
Free amino acids in the blood of man and animals: V. Effects of myleran, dimethylmyleran and related compounds in chronic granulocytic leukemia by G. ROUSER, K. KELLY, B. JELINEK AND D. HELLER (Duarte, Calif.) . . . . .	413
Free amino acids in the blood of man and animals: VI. Changes following glutamine ingestion by normal individuals and patients with chronic leukemia by G. ROUSER, K. KELLY, B. JELINEK, E. ROBERTS AND F. W. SAYRE (Duarte, Calif.) . . . . .	430
Free amino acids in plasma, brain and muscle following hepatectomy (Invited Discussion) by E. V. FLOCK AND J. L. BOLLMAN (Rochester, Minn.) . . . . .	449
Increase in urinary amino acids associated with pantothenic acid deficiency in the rat (In- vited Discussion)* by J. D. MARKS AND H. K. BERRY (Cincinnati, Ohio) . . . . .	461
Free amino acids in brain after administration of imipramine, chlorpromazine and other psychotropic drugs (Invited Discussion) by H. H. TALLAN (Ardsley, N.Y.) . . . . .	465
A survey of the amino acids and related compounds in nervous tissue (Invited Discussion)* by H. H. TALLAN (New York) . . . . .	471
Free amino acids in brain after treatment with psychotropic drugs (Invited Discussion) by E. MUSSINI AND F. MARCUCCI (Milano, Italy) . . . . .	486
The effect of psychotropic drugs and chemically related substances on $\gamma$ -aminobutyric acid and glutamic acid in brain tissue (Invited Discussion) by M. J. E. ERNSTING, W. F. KAFOE, W. TH. NAUTA, H. K. OOSTERHUIS AND P. A. ROUKEMA (Amsterdam, The Netherlands) . . . . .	493
Effects of 4-methoxymethylpyridoxine and carbonyl trappings agents on amino acid content of mammalian brain and other tissues (Invited Discussion) by C. F. BAXTER AND E. ROBERTS (Duarte, Calif.) . . . . .	499
Discussion Chairman: G. ROUSER . . . . .	509

#### VII. Round Table Discussion

Methodology and occurrence of free amino acids Edited by M. WINITZ . . . . .	512
---	-----

### PART TWO — DYNAMIC ASPECTS OF CELLULAR FREE AMINO ACID POOLS

#### I. Permeability and amino acid transport

On the mechanism of amino acid transport into cells by H. N. CHRISTENSEN, H. AKEDO, D. L. OXENDER AND C. G. WINTER (Ann Arbor, Mich.) . . . . .	527
Some remarks on active transport and exchange diffusion of amino acids in Ehrlich cells (Invited Discussion) by E. HEINZ (Frankfurt a/M, Germany) . . . . .	539

\* Invited discussion not presented at the conference.



Uptake of tyrosine by brain <i>in vivo</i> and <i>in vitro</i> (Invited Discussion) by G. GUROFF AND S. UDENFRIEND (Bethesda, Md.) . . . . .	545
Cerebral passage of free amino acids (Invited Discussion)* by A. LAJTHA (New York) . . . . .	554
Discussion Chairman: H. CHRISTENSEN . . . . .	564
Transport and accumulation of amino acids by microorganisms by J. T. HOLDEN (Duarte, Calif.) . . . . .	566
The mechanism of amino acid pool formation in <i>E. coli</i> by R. BRITTEN AND F. T. McCLURE (Washington D.C.) . . . . .	595
Some properties and potential uses of bacterial mutants defective in amino acid transport (Invited Discussion)* by M. LUBIN (Boston, Mass.) . . . . .	610
Discussion Chairman: H. CHRISTENSEN . . . . .	612

## II. Amino acid pool turnover

Limiting factors in biosynthesis of macromolecules by J. M. REINER (Atlanta, Ga.) . . . . .	617
Metabolic pools and the biosynthesis of protein by D. B. COWIE (Washington D.C.) . . . . .	633
The function and control of intracellular protein turnover in microorganisms by H. O. HALVORSON (Madison, Wisc.) . . . . .	646
Discussion Chairman: J. REINER . . . . .	655
Dynamics of amino acids in plants by S. ARONOFF (Ames, Iowa) . . . . .	657
The free nitrogen compounds in plants considered in relation to metabolism by F. C. STEWARD AND R. G. S. BIDWELL (Ithaca, N.Y.) . . . . .	667
Amino acid pools, protein synthesis and protein turnover in human cell cultures by H. EAGLE AND K. A. PIEZ (New York) . . . . .	694
Discussion Chairman: J. REINER . . . . .	706
The role of the liver and the non-hepatic tissues in the regulation of the blood free amino acids levels by L. L. MILLER (Rochester, N.Y.) . . . . .	708
<i>In vivo</i> compartments of glutamic acid metabolism in brain and liver by H. WAELSCH (New York City, N.Y.)* . . . . .	722
Discussion Chairman: H. EAGLE . . . . .	731
Free amino acids as obligatory intermediates in protein synthesis by R. B. LOFTFIELD (Boston, Mass.) . . . . .	732
Discussion Chairman: H. EAGLE . . . . .	738
Biochemical production of lipo-amino acid compounds by B. AXELROD, J. L. HAINING AND T. FUKUI (Lafayette, Ind.) . . . . .	742
On the metabolic importance of amino acid-lipid complexes by R. W. HENDLER (Bethesda, Md.) . . . . .	750
Discussion Chairman: H. EAGLE . . . . .	759

## III. Round Table Discussion

State of the intracellular amino acids Edited by E. HEINZ . . . . .	762
Author Index . . . . .	778
Subject Index . . . . .	779

\* Invited contribution not presented at the conference.

## INTRODUCTION TO CONFERENCE

E. ROBERTS

*Department of Biochemistry, City of Hope Medical Center, Duarte, Calif. (U.S.A.)*

To date, most of the effort of the biochemist has been devoted to the description of the properties of the chemical substances present in living matter. The list is far from complete, as evidenced by the daily appearance of reports dealing with the characterization of new molecular entities of biological interest. Although it has been recognized that substances can pass in and out of living systems and can interact and be transformed in these systems in manners potentially explicable by principles of physics and chemistry, in no single instance have the *details* of these processes been described adequately for a *given* substance in a particular type of living cell. Although biochemistry had its beginnings as a handmaiden to medicine in attempting to correlate manifestations of disease processes with gross chemical changes in blood, urine and tissue, in recent years the biochemist has joined with the cytologist and microbiologist in combining knowledge and techniques in the study of the chemistry and function of parts of various cells, *viz.*, membrane, nucleus, mitochondria, microsomes, etc. However, these efforts have not yet answered the question: How do all of the verifiable observations add up to what one observes as life?

Some of the key questions still to be answered relate to the properties of the bricks and mortar of life, the lipids, polysaccharides, proteins, the desoxy- and ribonucleic acids, and various complexes and mixtures of these materials. These substances are formed largely from smaller molecules by hereditarily determined processes resembling directed polymerization rather than by random assembly of smaller units. The cellular chemistry of even the smaller units is incompletely known.

The point of departure chosen for this conference was the discussion of free or easily extractable amino acids and related substances in living cells, small molecules which are not only the building blocks of proteins, but also which have myriad other functions within cells. Not only is it of interest to know the quantity of an amino acid in a given amount of a particular tissue or cell type, but it is of ultimate interest to be able to determine the past history, present position, and future fate of a particular molecule in a specific cell. To our knowledge the information relevant to these problems which has been accumulated to date in various laboratories throughout the world has not been summarized in one place so that an adequate perspective could be attained. We are fortunate in having present a number of people who have contributed importantly to this field and look forward to an exciting exchange of information.



*PART ONE*

OCCURRENCE OF FREE  
AMINO ACIDS



## I. ANALYTICAL METHODS

### IDENTIFICATION OF THE ELUSIVE AMINO ACID

MILTON WINITZ

*Laboratory of Physiology, National Cancer Institute, National Institutes of Health,  
Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Md. (U.S.A.)*

#### CRITERIA FOR ACCEPTANCE OF NATURAL OCCURRENCE OF AN $\alpha$ -AMINO ACID

In 1931, in a brilliant review entitled *The history of the discovery of the amino acids*, VICKERY AND SCHMIDT<sup>1</sup> proposed certain criteria that should be met before any report of the existence of a new amino acid as a constituent of proteins be generally accepted as valid. These criteria are given in what follows:

1. In order that an amino acid shall be accepted as a definite product of the hydrolysis of proteins, it must also have been isolated by some worker other than its discoverer.

2. Its constitution must have been established by synthesis and by demonstration of identity between the synthetic product and the natural racemized product, or by actual resolution of the synthetic product and preparation of the optically active natural isomer.

3. The substance must be liberated by hydrolysis from a preparation of a protein of demonstrated purity and must be adequately characterized by analysis of salts and of typical derivatives.

The protein-derived amino acids selected by VICKERY AND SCHMIDT, in 1931, which were acceptable according to these criteria, included all of those presently believed to occur in proteins with the exceptions of threonine, hydroxylysine, asparagine, glutamine and triiodothyronine, which had not yet been discovered, and included too  $\beta$ -hydroxyglutamic acid<sup>2, 3</sup>. Some 2 years later, SCHMIDT<sup>4</sup> recommended that norleucine be added to the list of accepted amino acids because of its apparent conformity with all of the required criteria. However, the seemingly irrefutable evidence upon which the acceptance of both  $\beta$ -hydroxyglutamic acid and norleucine rested was subsequently shown to be so tenuous that neither, at the present time, receives serious consideration as a constituent of proteins<sup>5</sup>. Such exceptions notwithstanding, the VICKERY-SCHMIDT criteria have served an invaluable role in that they prompted a generally more critical, as well as a more conservative attitude toward the acceptance of a new amino acid. Thus, amino acids such as diaminoglutaric acid<sup>6, 7</sup>, hydroxyaspartic acid<sup>6, 7</sup>, sarcosine<sup>8</sup>,  $\alpha$ -amino adipic acid<sup>9</sup>, norvaline<sup>10, 11</sup> and a host of others have, at one time or another, been reported to occur in proteins, but such claims either have subsequently been proven questionable or have not yet received the experimental support necessary for their general acceptance.

In addition to the amino acids which are known constituents of proteins, a number of amino acids have been found to occur in nature, either in the free state or in chemical combination in non-protein compounds of varying molecular size. Such amino acids have been detected in the biological fluids and tissues of various plants and animals, in the circulatory system, among the products of excretion, as inter-

mediates in the metabolic processes, as components of antibiotics and as bacterial decomposition products. Reports in the chemical literature allude to well over a hundred different amino acids of non-protein derivation that have been isolated from such sources<sup>12</sup>. That they may be possessed of a most diverse composition and structure is indicated by the few examples shown in Fig. 1. Although some of these amino acids have been unequivocally established as occurring in nature by a strong body of experimental data, somewhat more have been supported by data that would, at best,

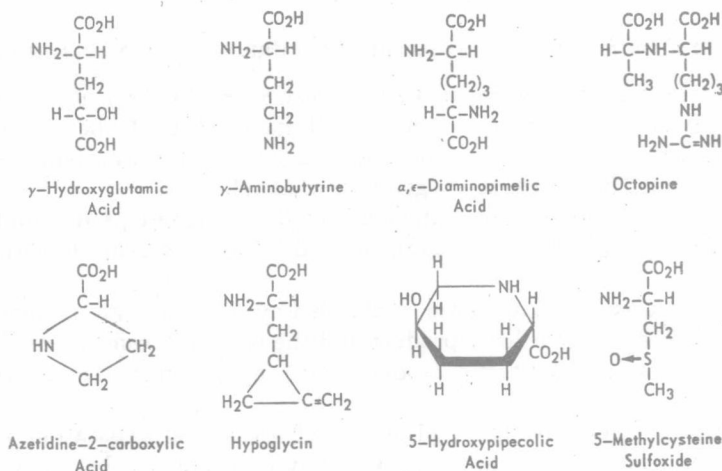


Fig. 1. Some naturally occurring amino acids of non-protein derivation.

provide only strong presumptive evidence for their natural occurrence. With most of the amino acids, however, sufficient evidence has not yet been amassed to demonstrate their existence in nature with even a modicum of assurance. These considerations, in addition to the appearance of a continually increasing number of reports of new amino acids of both the D- and L-varieties in nature, point out the urgent need for criteria which, if met, would reliably establish the natural occurrence of these amino acids. Proposals for such criteria are offered below:

1) The new amino acid should be physically isolated in sufficient quantity, and purified by several crystallizations from water, water-alcohol, or some other suitable solvent system, in order to obtain accurate and meaningful carbon, hydrogen, nitrogen and other possible elemental analyses.

2) Chromatographic procedures, whether two-dimensional techniques on paper employing different solvent systems, or elution techniques with columns employing various solvent mixtures, should show it to possess a single spot or peak, respectively, in order to assure the possibility of molecular homogeneity.

3) The molecule should be degraded by chemical organic procedures or unequivocal enzymatic procedures to identifiable products or fragments in order to obtain a knowledge of its structure.

4) Verification of the structure ultimately assigned to the molecule should be achieved through synthesis of the material employing unequivocal chemical procedures.

5) The synthetic material should be resolved into its optical antipodes and the

physical, chemical and biological properties of the antipodes so secured compared with those of the natural material; in the case of amino acids with more than one asymmetric center, separation of the diastereomers should precede the resolution of each racemate into its optical antipodes.

6) Determination of the optical configuration of the molecule should be accomplished by any of a number of chemical, physical, optical or biological techniques.

7) The natural occurrence of the new amino acid should be independently confirmed by some investigator other than the discoverer.

#### IDENTIFICATION BY CHROMATOGRAPHIC AND COLORIMETRIC MEANS

At the present time, a facile and convenient means for establishing the identity of an amino acid from natural sources involves a comparison of its chromatographic behavior with reference amino acids in a variety of solvent systems. Ofttimes, no other criteria are employed. Now it is well known that certain structurally related amino acids, such as leucine and isoleucine, reveal a chromatographic behavior in numerous solvent systems wherein appreciable differences in mobility are the exception rather than the rule. It is therefore evident that through the use of chromatographic procedures alone, the danger is ever present that an amino acid of as yet unsuspected structure will remain hidden behind, or occupy the same position as a closely related amino acid of known structure, and hence will be overlooked. This probability was dramatically emphasized only recently with the announcement by OGLE, LOGAN AND ARLINGHAUS<sup>13</sup> that a new imino acid had been isolated from acid hydrolysates of collagen, a protein that had hitherto been the subject of chromatographic analysis by many investigators in many laboratories. In a private communication from Dr. OGLE, it was learned that during a study of the peptide sequence of collagen, a tripeptide was isolated which, after acid hydrolysis, displayed a previously unobserved peak running just before hydroxyproline upon passage of the hydrolysate through a Dowex-50 column employing 0.2 N sodium phosphate buffer as the eluant and a pH of 3.1. Analysis revealed that the new material was an imino acid and was of the same elemental composition as  $\gamma$ -hydroxyproline. That the compound was neither proline nor  $\gamma$ -hydroxyproline was indicated by a comparison of infrared spectra and the melting points of various derivatives, by the color reaction obtained with ninhydrin in glacial acetic acid and by the fact that it failed to react with dimethylaminobenzaldehyde following oxidation with chloramine-T. As degradation of the material with permanganate yielded  $\beta$ -alanine, the new material was assigned the structure of  $\beta$ -hydroxyproline. *It should be noted that the paper-chromatographic behavior of this new material was identical with that of  $\gamma$ -hydroxyproline in all solvent systems tested.*

That amino acids of varying structure may possess identical mobilities in a number of different solvent systems is an occurrence that is by no means rare. One such occurrence that was noted in our laboratory<sup>14</sup> some years ago involved a material which was obtained from the copper-catalyzed condensation of pyruvic acid and glycine in an alkaline medium (Fig. 2), and which possessed an empirical formula of  $C_8H_9NO_5$ . This formula could correspond to  $\beta$ -hydroxy- $\beta$ -methylaspartic acid if it were assumed that the condensation involved the  $\alpha$ -carbon atom of the pyruvic acid reactant. If, however, the condensation involved the  $\beta$ -carbon atom of the pyruvic acid, then



$\gamma$ -hydroxyglutamic acid, which possesses the same empirical formula, could result. As authentic samples of  $\gamma$ -hydroxyglutamic acid were available, a chromatographic comparison of this material with the unknown synthetic material was made in three different solvent systems. The  $R_F$  values were identical in every instance. However, it was noted that in contradistinction to the usual rapid purple color given by  $\gamma$ -hydroxyglutamic acid with the ninhydrin reagent, the unknown synthetic product exhibited some unique color reactions. Thus, when a solution of this material was spotted on filter paper and the paper sprayed with a solution of ninhydrin in acetone and subsequently heated, a bright yellow spot appeared. The yellow color gradually changed to gray-brown after 4–8 h, and finally to purple after 18–24 h. If, however,

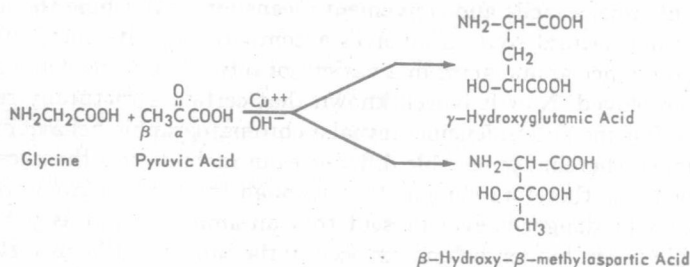


Fig. 2. Potential products arising from copper-catalyzed condensation of pyruvic acid and glycine.

the paper was sprayed with a basic ninhydrin solution and heated, the yellow spot appeared but almost immediately turned purple. Such behavior indicated that the new material was other than the suspected  $\gamma$ -hydroxyglutamic acid. Had it, however, responded to ninhydrin in the usual manner, it might have been all too tempting, on the basis of its mobility behavior alone, to assign it the erroneous structure.

From what has already been said, it becomes readily apparent that visual observation of the color-staining process on paper will sometimes permit distinctions between amino acids that cannot be made on the basis of movement analysis alone. A few instances of the specific color reactions revealed by the different amino acids after development with various color-inducing reagents are listed in Table I. Thus, it is commonly known that whereas most amino acids exhibit a blue or reddish-purple color after treatment with the ninhydrin reagent, proline displays a yellow color, tyrosine gives a dull greenish-purple, hydroxyproline yields a brown-yellow color (which serves to distinguish it from the nearby alanine spot<sup>15</sup>), glycine shows a rather grayish-purple color, asparagine reveals an orange-brown color (which serves to distinguish it from the nearby glycine spot<sup>16</sup>), and aspartic acid is characterized by a rather bright blue. Treatment of the paper chromatogram with weak alkali intensifies the blue color of the ninhydrin spot of phenylalanine<sup>17</sup>, whereas treatment of the paper with cyclohexylamine prior to spraying with ninhydrin leads to a blue color for aspartic acid, orange for cystine, grayish-green for histidine, bluish-gray for phenylalanine, yellow for proline, carmine for hydroxyproline, grayish-purple for threonine, gray for tyrosine, and reddish-brown for glycine, with all of the other protein-derived amino acids yielding the more usual purple color<sup>18</sup>. In addition, a variety of other staining reagents are available which are more or less specific for certain amino