COMPARATIVE BIOCHEMISTRY

A Comprehensive Treatise

Edited by *MARCEL FLORKIN*

HOWARD S. MASON

Volume V

CONSTITUENTS OF LIFE   Part C

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Volume V

CONSTITUENTS OF LIFE—PART C

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PREFACE

In order to provide a systematic comparison of the biochemical phenomena of life throughout the phylogenetic scale, Comparative Biochemistry has been organized as follows: Volume I and II are primarily concerned with the biological transformations of energy; Volumes III, IV, and V with the biological transformations of matter; and Volumes VI and VII with the organized systems occurring in living organisms.

The present volume completes the section on Constituents of Life. It contains chapters on the structure and distribution of amino acids, on the distribution and metabolism of inorganic nitrogen compounds, on acid metabolism (the citric acid cycle and other cycles), and on the comparative biochemistry of halides. A number of chapters in Volume IV dealt with special forms of adaptation of protein structure: keratin, sclerotized proteins, silk, and protein transformation during the coagulation of blood and during amphibian metamorphosis. Another form of adaptation of protein structure, collagen, is dealt with in the present volume. This completes the section, composed by Volumes III, IV, and V, and devoted to the principal classes of constituents of cells and organisms, their distribution, and the comparative enzymology of their biogenesis and metabolism. The decision not to postpone the printing of chapters already in hand has forced the editors not to follow exactly the original plan of organization, as far as the sequence of chapters is concerned. The editors are glad to have been able to bring together the material contained, as planned, in Volumes III, IV, and V, and are confident that any inconvenience caused by the disarrangement of the original sequence will be corrected by a perusal of the general index which will appear at the end of Volume VII.

In the Preface to Volume I of this treatise, the editors expressed regret at not having been able to include in that volume, which dealt with the sources of free energy, the chapter on photosynthesis; this chapter is included in the present volume.

As in the case of previous volumes, the publishers have provided us with prompt, competent, and reliable assistance. We wish again to express our gratitude to them.
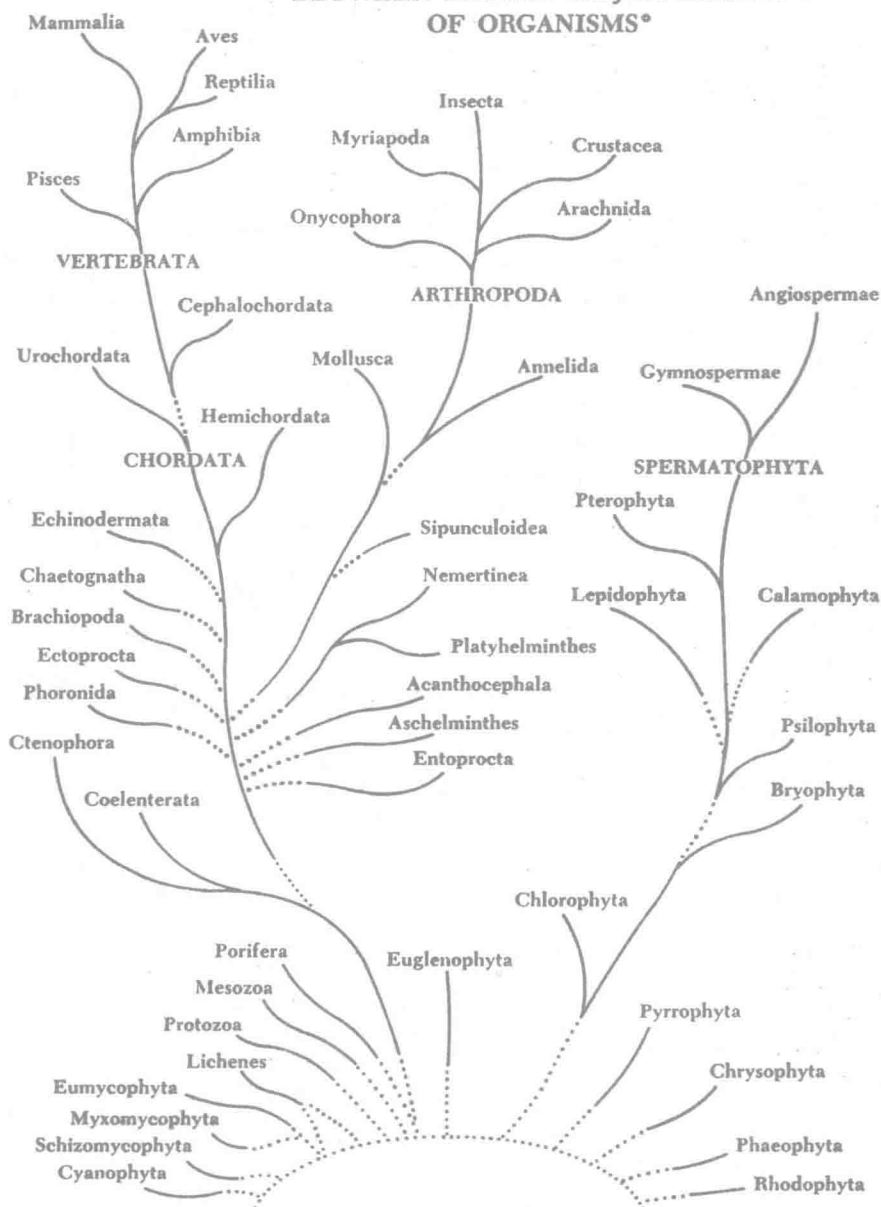
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February, 1963

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Mammalia

Aves

Reptilia

Amphibia

Insecta

Myriapoda

Crustacea

Pisces

Onycophora

Arachnida

VERTEBRATA

Cephalochordata

ARTHROPODA

Angiospermae

Urochordata

Mollusca

Annelida

Gymnospermae

Hemichordata

CHORDATA

SPERMATOPHYTA

Echinodermata

Pterophyta

Sipunculoidea

Chaetognatha

Nemertinea

Brachiopoda

Lepidophyta

Calamophyta

Ectoprocta

Platyhelminthes

Phoronida

Acanthocephala

Psilophyta

Ctenophora

Aschelminthes

Entoprocta

Bryophyta

Coelenterata

Chlorophyta

Porifera

Euglenophyta

Mesozoa

Pyrrophyta

Protozoa

Lichenes

Chrysophyta

Eumycophyta

Myxomycophyta

Schizomycophyta

Phaeophyta

Cyanophyta

Rhodophyta

xi

CHART II: ANIMAL KINGDOM

Divisions	Estimated Number of Species[d]	Taxonomic Classifications
Protozoa (acellular animals)	15,000	
Mesozoa	—	
Porifera (sponges)	5,000	
Coelenterata (coelenterates)	10,000	
Ctenophora (comb jellies)	100	} Radiata
Platyhelminthes (flat worms)	6,000 }	
Nemertinea (nemertine worms)	500 } Acoelomates	
Aschelminthes[a] } Acanthocephala[a] }	7,000 } Pseudocoelomates	
Entoprocta[b] Ectoprocta[b] (moss animals) }	3,000	
Phoronida	15	} Protostomia
Brachiopoda (lamp shells)	120	
Mollusca (mollusks)	70,000	} Schizocoela
Sipunculoidea	—	
Annelida[c] (segmented worms)	6,500 } Eucoelomates	
Arthropoda (arthropods)	750,000	} Bilateria
Chaetognatha (arrow worms)	30	
Echinodermata (echinoderms)	5,000	} Enterocoela } Deuterostomia
Hemichordata Chordata (including vertebrates) }	60,000	

[a] Includes Rotifera, Gastrotricha, Kinorhyncha, Nematoda, Nematomorpha, Priapuloidea. Formerly called Nemathelminthes.

[b] Formerly in Bryozoa.

[c] Includes Echiuroidea.

[d] Taken from "Handbook of Biological Data" (4), p. 533.

CHART III: PLANT KINGDOM

Divisions	Estimated Number of Species[d]	Major Synonymous Terms			
Euglenophyta (euglenoids)	340				
Chlorophyta (green algae)	5,700				
Pyrrophyta (cryptomonads, dinoflagellates)	1,000				
Chrysophyta (yellow green algae, diatoms)	5,700	Algae			
Phaeophyta (brown algae)	900				
Rhodophyta (red algae)	2,500		Thallophyta		
Cyanophyta[a] (blue-green algae)	1,400				
Schizomycophyta[a] (bacteria)	1,300[e]				Cryptogamia
Myxomycophyta (slime molds)	430	Fungi			
Eumycophyta (true fungi)	74,000				
Lichenes (lichens)	15,500				
Bryophyta (mosses and liverworts)	23,800		Bryophyta		
Psilophyta[b] (whisk ferns)	3	Psilopsida			
Calamophyta[b] (horsetails)	30	Sphenopsida			
Lepidophyta[b] (lycopods)	1,300	Lycopsida		Tracheophyta	
Pterophyta[b, c] (ferns)	10,000	Pteropsida			
Spermatophyta (seed plants)	201,000				Phanerogamia

[a] Sometimes grouped as Schizophyta.
[b] Formerly classed as Pteridophyta.
[c] Formerly classed as Filicineae in Pteropsida.
[d] Taken from "Handbook of Biological Data" (4), p. 533.
[e] There is much disagreement concerning designation of species here.

CONTENTS

CHAPTER 1

Amino Acids: Structure and Distribution *

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I. Introduction

The first-known amino acids, asparagine and cystine, were discovered in the years 1806 and 1810, respectively. The research that ensued was directed primarily toward proteinogenous amino acids, investigation of which seems, in a sense, to have come to a close. In 1951, Grassman *et al.* (*281*) wrote: "Von den ungefähr 40 beschriebenen sogenannten natürlichen Aminosäuren sind nur 23 sicher als Eiweissbausteine nachgewiesen." Today we can assume a figure of about 120 for amino acids in general, excluding related compounds. This figure is continually growing, as shown in the graph (Fig. 1). The total number of known proteinogenous acids, on the other hand, has not increased further since 1940. To be sure, a number of notable amino acids are contained in peptides, particularly in lower plants, which have as yet not been found free but which cannot be described as actual protein constituents.

The rapid advance in our knowledge is to be attributed, in the first place, to newer analytic methods—particularly paper chromatography, which has considerably aided in the discovery of as yet unidentified

* Translated from the German by Dr. Herbert Heineman, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

substances. Conversely, progress in methodology has stimulated the search for new substances. By far the greater part of plants and animals is still unexplored. Every systematic advance in any taxonomic group in recent years has drawn attention to the existence of new and still unidentified amino acids. Particularly rich has been the yield in the realm of plant chemical investigation.
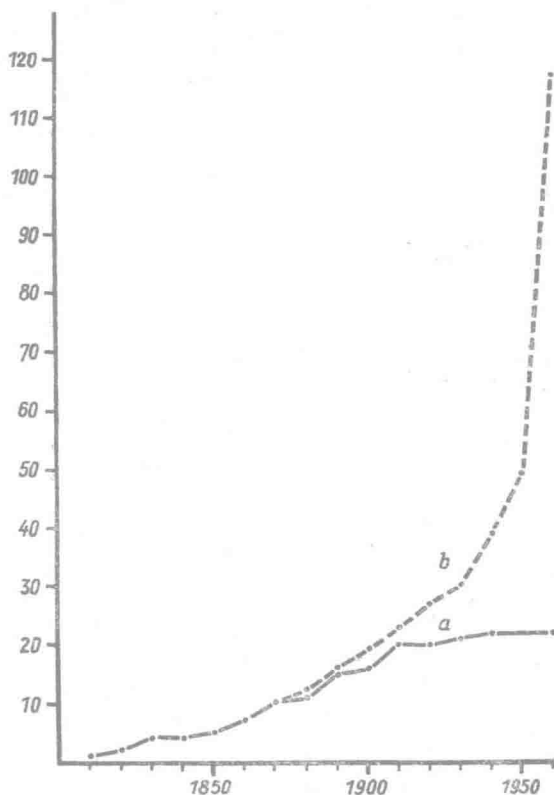
FIG. 1. Growth in the number of known amino acids. Curve *a*, amino acids occurring in proteins; curve *b*, amino acids occurring in free form.

It is known that higher animals and humans are unable to synthesize a number of proteinogenous amino acids essential to life. The fact must not be overlooked that in the realm of heterotrophic plants, especially the fungi and bacteria, there also frequently exists a nutritional need for particular amino acids. The typically autotrophic plant, particularly the higher plant, cannot exist in the form of amino acid-deficient mutants that doubtless are continually arising.

The plant thus represents the first manifestation of far-reaching

chemical performance. Beyond this, however, it produces a large number of substances, presumably unimportant to life, that in general are not made by animals. These include many "secondary" plant substances of types such as alkaloids, betaines, etc. Whether the presence of these substances is primary evidence of greater capacity for chemical achievement, or whether it is to be attributed to lack of a constantly functioning excretory system, is open to question. In animals the typical end products of metabolism are generally completely eliminated. In plants, at least they remain in vacuoles, where they are afforded further exposure to the enzymatic apparatus of the protoplasm, leading to secondary changes and finally to biochemical stability.

Thus plants and animals appear to be chemically very different. Yet research in the past decade has shown that the basic metabolic pathways are similar in all organisms, to the extent that even the enzymes correspond to one another. From this alone the great similarity in the composition of plant and animal proteins is evident. This holds true for metabolically active proteins. Among reserve proteins, the structural proteins, there is a wealth of differences.

Such differences also exist for the soluble storage forms of nitrogen ("detoxified ammonia"), which play a great role in the life of the plant; the latter is, by virtue of its autotrophy, capable of resynthesizing the breakdown products of proteins and nucleic acids, and its entire existence, in fact, depends on its strict economy of metabolism. Any plant that excreted as urea or uric acid the ammonia constantly being formed in protein degradation would succumb to nitrogen depletion. For despite the greatest metabolic economy in nitrogen, the latter is to a large extent a limiting factor in the productivity of plants. In animals, therefore, we find numerous interesting amino acids in excreta, particularly urine.

In plants such compounds are stored. Among such storage forms of nitrogen that occur in large concentrations in plants are asparagine, glutamine, citrulline, arginine, acetylornithine, allantoin, and alanine. They may amount to 90% of soluble nitrogen in the storage depots, for example in tubers, roots, bulbs, stems, and fruits. In dry seeds such amino acids are not prominent. Almost all of them are in a position to be incorporated directly or indirectly into protein synthesis. There are instances, however, as in canavanine, in which amino acids accumulate in the seeds, evidently because during ripening they cannot enter into the synthesis of proteins or their building blocks. A large number of interesting and physiologically quite active amino acids have in recent years been found as "mass substances" in such storage depots even of lower plants.

While some of these compounds enjoy universal distribution, some apparently are limited in their occurrence to a few plants or groups of plants. For this reason, repeated search has been made for a relationship between the occurrence of particular amino acids and the taxonomic position of a plant. Such considerations are of the greatest interest, since they would permit conclusions regarding phylogenetic connections. To date, however, no instances have been found of such an occurrence limited to particular plants or groups of plants. Even in the best example among amino acids, acetidine-2-carboxylic acid, an unequivocal relationship between its occurrence and the taxonomic position of the plant that contains it cannot be made (243). Similar observations were made by us while investigating the occurrence of canavanine in Papilionaceae (732).

A frequent observation is the occurrence of chemically related compounds in the same plant species. Examples are the occurrence of baikiaine, pipecolic acid, and 5-hydroxypipecolic acid in *Baikiaea;* of proline, methylproline, and hydroxymethylproline in apples; or of various glutamic acid derivatives in tulips and in the ferns *Adiantum* and *Phyllitis.* In only a few cases, however, have the physiologic connections of this occurrence been made clear, and the possibility remains that chemically related structures are formed by entirely different pathways. The latter possibility is suspected from investigations into the formation of glutamic acid derivatives.

Whereas some compounds are found in all parts of a plant, others are limited to particular parts or developmental stages. In various plants, for example, amino acids foreign to the seed appeared only during germination, as described by Done and Fowden (185) for γ-methyleneglutamine and γ-methyleneglutamic acid in *Arachis hypogaea,* and by Virtanen *et al.* (761) for homoserine in *Pisum sativum.*

Our knowledge regarding the role of these compounds, particularly the newly discovered amino acids, in the nitrogen metabolism of individual plants is still very meager. For not in all cases is it easy to establish a function such as nitrogen storage, as one can for compounds that occur in large quantities. Since, moreover, but very little has become known about the biosynthesis and degradation of compounds only incompletely identified and partly distinguished by particular structural characteristics, it is hardly possible to estimate their significance for any single plant.

Apart from such noteworthy taxonomic and ontogenetic peculiarities, consideration must be given to the influence of environmental conditions, climate, and nutrition on the qualitative and quantitative composition of the amino acid mixture, especially in higher plants. Thus, in *As-*

plenium, Bramesfield and Virtanen (*85*) were able, in two consecutive climatically different years, to demonstrate γ-hydroxy-γ-methylglutamic acid in one year but not in the next. Steward and co-workers (*683a*) investigated soluble nitrogen in *Mentha piperita,* a long-day plant, and found the amino acid spectrum strongly dependent on environmental conditions. Thus under long-day conditions very little asparagine was formed, although aspartic acid was present. In contrast, there was much glutamine in association with glutamic acid. Under short-day conditions glutamic acid is markedly reduced, and together with glutamine asparagine is found primarily in the stem. These investigations also bring to light the great difference in composition of the amino acid mixture in leaves, stems, and roots. Lack of potassium, and to some extent also of calcium, promotes the formation of asparagine. Lack of sulfur leads to a marked synthesis of arginine, especially under long-day conditions. Reference is also made to the investigations of Champigny (*127*) on the influence of light upon the synthesis of amino acids in *Bryophyllum.* It is impossible to go into the many references in the literature to the variability of amino acids in the same species, in particular since it is not generally established whether the claims have universal validity or only represent special reactions of a particular organism.

In view of the great significance of lower organisms in comparative biochemistry, a few statements must be made regarding algae and fungi. Algae resemble the higher plants. Occasionally such rarely observed compounds as taurine, *N*-methyltaurine, di-*N*-methyltaurine, citrulline, ornithine, and also iodine-containing amino acids make their appearance. Marine algae apparently accumulate peptides frequently, for example, according to Dekker *et al.* (*172*), fastigiatine, a L-pyrrolidonyl-α-L-glutaminyl-L-glutamine, in *Pelvetia fastigiata.* It is not quite certain whether the pyrrolidinone ring is actually present in the living cell. Other peptides, in addition to glutamic acid, also contain aspartic acid (*666*). Perhaps this peptide formation prevents the diffusion of the acid form of the amino acid into the alkaline sea water. Haas *et al.* (*296*) has presented the likelihood that these peptides are nitrogen storage substances, which are used up with the beginning of further growth.

Of a different physiologic type are a number of peptides in higher fungi. *Amanita phalloides* contains the following cyclic peptides: the amanitins, phalloin, and phalloidin, which have been investigated particularly by Th. Wieland (*808*). In these compounds a series of amino acids have been found, which, in their structure and optical configuration, differ from the naturally occurring ones hitherto known. Thus hydrolysis of phalloidin yields cysteine, α-hydroxytryptophan, alanine,

and allohydroxyproline. Peptides bound to lysergic acid are also formed among the alkaloids of ergot. Here α-hydroxy-α-amino acids seem to play a role. In this group of peptides composed of anomalous amino acids, in the broad sense, belong also the putrefaction toxins of *Fusarium* species, the aspergillic acid of *Aspergillus flavus*, the penicillins, etc. The latter contain D-amino acids. These acids occur also in the peptides of some bacteria, whereas their proteins throughout resemble those of higher plants. These D-amino acids also frequently enter into cell walls and capsules; they are often constituents of antibiotics, which on the whole are distinguished by anomalous amino acids.

II. Proteinogenous Amino Acids

A. ALIPHATIC AMINO ACIDS

1. *Glycine (Glycocoll, Aminoacetic Acid)*

$$CH_2-COOH$$
$$|$$
$$NH_2$$
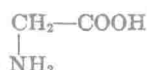
Glycine is the simplest of all protein constituents: it contains no asymmetric carbon atom. It was obtained from gelatin in 1820 by Braconnot (*84*) through hydrolysis with sulfuric acid. It thus became the first amino acid to be derived in pure form from a protein hydrolyzate. Cahours (*113*) succeeded in elucidating its structure in 1857 and in synthesizing it from monochloroacetic acid and ammonia in 1858. At about the same time the compound was synthesized by Perkin and Duppa (*546*).

Shorey in 1897 (*659*) was the first to isolate glycine from a plant source, obtaining it from sugar cane in concentrations of 0.1–0.4%. In addition to the sporadic occurrence of free glycine in higher plants (*331*), its presence has been proved in mammalian organs (*407*). In combined form it is found in practically all proteins (*41, 686, 730*). Particularly large quantities are present in collagen (*505*) and in silk fibroin (*64*). Plant proteins only rarely contain more than 0.2–1.0% glycine.

Glycine occupies a central position in metabolism. This fact is already apparent from the variety of pathways leading to its formation. Among these reactions are: the amination of glyoxylic acid; the demethylation of sarcosine; the degradation of threonine to glycine and acetaldehyde; and, no less important, the physiologic interconversion between glycine and serine, the mechanism of which has recently been thoroughly investigated (*64, 88, 314, 466*).

Glycine is involved in the synthesis of a series of compounds of physiologic interest. In animals, reaction with benzoic leads to the formation of hippuric acid (626). This predominantly renal mechanism

$$\text{C}_6\text{H}_5-\text{CO}-\text{NH}-\text{CH}_2-\text{COOH}$$
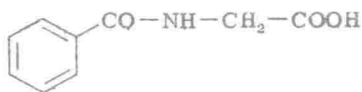
Hippuric acid

serves to excrete the benzoic acid contained in food. In glycocholic acid glycine exists in combination with cholic acid. The incorporation of glycine into the tripeptide glutathione was observed by Braunstein *et al.* (86). The amino acid takes part in the second step in this reaction, being coupled to the previously formed L-γ-glutamylcysteine (807). In purine synthesis, glycine contributes to the molecule carbon atoms 4 and 5 as well as nitrogen atom 7. This incorporation involves coupling to 5-phosphoribosylamine with the formation of glycinamide ribotide
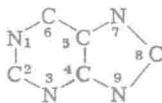
(glycine-N-amino-5'-phosphoribotide) (104). Participation of glycine in the synthesis of the protoporphyrin molecule is limited to the α-carbon atom and the nitrogen atom, since the carboxyl group is split off during the formation of δ-aminolevulinic acid from α-amino-β-ketoadipic acid (415; 657).

Sarcosine is formed from glycine by methylation. This compound was first obtained by von Liebig (429) in the course of the decomposition of creatine. Elucidation of its composition as well as synthesis was attained by Volhard in 1862 (789).

$$\text{CH}_2-\text{COOH}$$
$$|$$
$$\text{NH}-\text{CH}_3$$
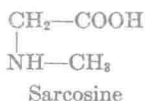
Sarcosine

Sarcosine, the natural occurrence of which was first described by Kossel and Edlbacher (401), is a constituent of muscle and is also found in the organs of echinoderms. Haworth and co-workers (315) isolated it from a protein hydrolyzate of *Arachis hypogaea*. They obtained 2–3 mg. sarcosine from 7.5 gm. protein. Free sarcosine was found by Virtanen and co-workers (434) in *Cladonia silvatica*. Additional sources of this compound have been repeatedly described (528, 708) in recent times.