THE METABOLISM OF PROTEIN CONSTITUENTS in the Mammalian Body

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PREFACE

THE past fifteen years have brought a development of biochemical research on an unprecedented scale. Much of this the scientific world owes to the application of isotopic tracers, and no branch of biochemistry has profited more from this technique than the intermediary metabolism of nitrogenous compounds. Another causative factor for this development was the knowledge gained in the field of enzyme chemistry, which greatly aided the elucidation of the individual metabolic steps.

The last two comprehensive works on the biochemistry of protein constituents were published before the beginning of this new era. I am referring to Mitchell and Hamilton's lucid account of the biochemistry and chemistry of amino acids in the Monograph series of the American Chemical Society (1929), and also to the little-known, but monumental chapter of O. Neubauer on 'intermediary protein metabolism' in Volume V of the *Handbuch der normalen und pathologischen Physiologie* (Springer, 1928). Since then a wealth of new literature, amounting to several thousand experimental and review articles on protein metabolism, has filled the ever-increasing number of biochemical journals. The widening of the scope of biochemical research to include the services of physicists, mathematicians, and pathologists, side by side with biologists and chemists, has increased the difficulties of mastering the subject.

The task of sifting this flood of information was not undertaken lightheartedly. The intention was not to write another review on the subject but rather to tell a coherent story of the development of the various problems which present themselves in the metabolism of amino acids. In doing so, I had to limit my efforts to metabolism proper, as distinct from the chemistry; furthermore, I restricted my attention to the mammal, except for occasional illustrations taken from more primitive vertebrates.

An attempt to correlate divergent opinions on such problems made it necessary to emphasize concepts which, rightly or wrongly, appeared to me the most plausible. While experimental data supporting opposing views are given in every case, I have concentrated on conveying to the reader my own interpretation, rather than on simply enumerating the facts. For 'such lack of impartiality' I hope I may be forgiven; it should help scientists working on the fringe of the field to grasp the problems more readily, yet it should not prevent those more familiar with the subject from weighing critically my arguments.

The present volume of the book is concerned with ten protein con-

stituents as well as with related amino acids known to occur in the free state. I am at present preparing a further volume dealing with the remaining protein constituents. The fact that some of the amino acids described are involved in the synthesis of compounds of particular biological interest, or in chemical reactions of general importance, induced me to treat such compounds or reactions as separate entities. This led to a more detailed discussion on topics such as the formation of haem and uric acid, and on reactions like transmethylation and the oxido-reduction of tissue-SH groups.

Most of the material was collected during the sixteen years of my activities at the Biochemical School of Cambridge University. It was the inspiration instilled by Hopkins and the tradition carried on by his successors which encouraged me in my task. Hopkins's broad outlook has taught us to consider biochemical reactions as manifestations of the cellular organization rather than as individual chemical events. It is precisely this aspect which I have endeavoured to keep in the foreground.

Wherever possible, I have consulted authors of articles on which I have reported in this book. In this respect I recall with pleasure the stimulating conversations with members of Dr. H. T. Clarke's Laboratory at the Columbia University, New York. I am particularly grateful for the hospitality extended to me by Dr. V. du Vigneaud, Cornell University, New York, during my visits to his laboratory. I gladly seized the opportunity presented to me of completing the chapter on sulphur-bearing amino acids in this laboratory which has contributed so much to the subject.

It is a pleasure to thank my friends and colleagues of the Universities of Cambridge, London, and Bristol for reading selected sections as well as proofs. The bulk of the editorial work was undertaken by Dr. R. B. Fisher, Oxford, who has done much to improve the manuscript and to whom I wish to express my special thanks. The difficult task of preparing the Index of Subjects was undertaken by Dr. E. Watchorn, Cambridge, and that of the Index of Authors by Mrs. Gitta Arnold, London.

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I

INTRODUCTION

The metabolism of protein

As a constituent of the living matter protein occupies an outstanding position; it represents its most important structural element and cannot be replaced by any other biological material. Living matter, as well as inanimate material, undergoes changes imposed by the conditions existing in their surroundings. With inanimate material these changes result in permanent amendments to the physical structure or the chemical pattern. The living organism, on the other hand, has at its disposal a maintenance mechanism 'organized' by the directive forces of the 'organism', in the sense of Hopkins's prophecy in 1936 [28]: 'From a knowledge of individual events will proceed an understanding of the organisation of these events: that organisation which makes the organism.'

Through continuous replacement of their constituents the cellular structure of the tissues is preserved. In the resting state replacement keeps pace with wear and tear; in the growing phase the rate of the former exceeds the latter. It is surprising that this concept of the continuous replacement of the elements of the cellular material has come only as a relatively recent revelation to biochemists. In the view of many thinkers in other fields, it is precisely this process of continuous 'rejuvenation' of living matter which marks its distinction from the inanimate world.

'If . . . the aim of biochemistry is to describe life, at any level, in chemical terms, it may come more under the eye of philosophy than perhaps any other branch of biology' (Hopkins, 1936 [28]).

The interest of the scientist naturally centres on the nature of the mechanism by means of which such replacements are achieved by the organism. Similar problems occupied physiologists of earlier generations. According to their belief, replacement of the protein of living tissue by dietary amino acids takes place only to a very limited degree, such as is necessary to compensate for the 'wear and tear' of the body constituents. The remainder, i.e. the greater part, of the dietary material was believed to be catabolized and finally excreted in the urine (Folin [1, 2]). According to this concept the greater part of the protein structure had to be considered as metabolically inert material. Such was the accepted view till in 1935, when Borsook and Keighley's [3] experiments and their mathematical interpretations inspired the idea of a continuous synthesis and degradation of the cellular protein. 'When

protein is ingested, some of its amino acids contribute towards maintaining constant the concentration of free amino acids in the blood and tissues. A large fraction is synthesized into protein and peptides while the remainder is catabolized . . .' [26]. Finally, the isotope experiments of Schoenheimer and his associates secured convincing evidence of the incorporation of dietary amino acids and other dietary constituents into cellular material. On this Schoenheimer based his theory of the dynamic state of body constituents [4]. It is therefore evident that an important part of protein metabolism is concerned with the process of incorporating dietary amino acids into cellular peptide chains and then of releasing them. In this sense the metabolism of protein is synonymous with that of its constituents. The development of the isotope technique and particularly the widening of its scope through the introduction of radioactive and heavy carbon into biological tracer technique has firmly established the evidence for these over-all exchange reactions, though little is known with regard to their chemical details. It is possible that dietary amino acids are first degraded to small units which subsequently may find their way into the peptide chains. It is also conceivable that parts of the amino acid molecule, such as the amino group, are directly transferred to the peptide-linked protein constituents. In the latter case, the well-established processes of transamination or transamidation may provide the basis. It has been claimed that transamidation is catalysed by proteolytic enzymes [20], though a disparity has been found between the pH optima for hydrolysis and for transamidation in the action of several proteinases. Johnston *et al.* [20] make the interesting suggestion 'that, at physiological pH values, transamidation may represent a major reaction in *living* cells; upon *death* of the cells and the accompanying shift of pH to more acid values, the action of the proteolytic enzymes may be exclusively a hydrolytic one'. The source of energy for the exchange reactions must be sought in the catabolism of those protein constituents which, replaced by dietary components, are released into the tissue fluids. These resources are augmented by energy derived from the catabolism of carbohydrate, fat, and other biological constituents.

Thus, the metabolism of protein appears to consist of at least two principal phases. In the first phase, a large proportion of dietary protein constituents (or amino acids synthesized in endogenous reactions) are incorporated into tissue protein and a small proportion of dietary material is catabolized. The degree of intensity of incorporation differs considerably in the various organ proteins (Sprinson and Rittenberg [16]). In the second phase, the amino acids 'displaced' during the first phase undergo a multitude of metabolic changes. Their degradation may lead to the resynthesis of the same or a different type of protein constituent, to the formation of carbohydrate, fat, or other biological

material, or to the formation of excretory products. Dietary glycine, for instance, may during the first phase be found as the glycine component of tissue protein. The tissue-glycine so 'displaced' during the second phase may be converted to serine which then appears as a component of newly formed body protein, or the glycine may be incorporated into excretory products such as creatine or uric acid. The sulphur of dietary methionine may be detected either in the structure of the organ proteins or in urinary sulphate. A degradation, conversion, or resynthesis of amino acids within the protein structure is also feasible, though little is known about reactions of this type. Our present knowledge of the metabolic changes of protein constituents is essentially confined to reactions considered to occur while in a free state.

Free amino acids

The existence of a pool of free amino acids has been repeatedly claimed. From the results of isotope experiments its size has been calculated by Sprinson and Rittenberg [16] to be 0·5 g. nitrogen per kilo body-weight in human subjects. The level of the pool appears to be regulated by endocrinal action [17, 18, 19], and its distribution between cells and extracellular fluid has been studied. Christensen *et al.* [21] believe in the special role of glutamic acid in the regulation of the amino acid concentration within the cell and in extracellular fluids. A not inconsiderable proportion of amino acids escapes metabolic changes altogether and is excreted as such [22, 23, 24]. Since the excretion of amino acids is increased in dietary protein deficiency [25], it appears that the organism is unable in such cases to manufacture the specific amino acid pattern required for the synthesis of the tissue protein; consequently, residual protein constituents are excreted.

General reactions of amino acids

The basic structure of amino acids reveals certain features, in particular the α-amino groups which easily lend themselves to a metabolic attack. There are, however, cases known where the reactivity of the amino group is hindered by acylation as in the biological formation of N-acetylated amino acids [5]. With the majority of protein constituents the amino group can by transamination [27] be transferred intact to α-keto acids. In other cases direct deamination takes place [7]. The carboxyl group is less reactive and only a few cases of decarboxylation are known in mammalian tissue. The decarboxylation of serine to ethanolamine, as described in the chapter on serine, is a notable example. Decarboxylation of aromatic amino acids is another instance. The β- and γ-carbons and those still further removed from the amino group are more reactive than the carboxyl group. Their oxidation may

lead to aminodicarboxylic acids (as in the case of lysine) or to a shortening of the carbon chain, as in the conversion of serine into glycine.

Alanine $\mathrm{COOH.CH.CH_3}$ $\mathrm{NH_2}$

The biological reactions of alanine serve as an example of a comparatively simple type of amino acid metabolism. Pyruvic acid constitutes a significant metabolic stage in the degradation of the glucogenic amino acids. In the metabolism of alanine it is the only one of importance. A reviewer of this protein constituent could almost confine his task to the mere description of the removal of the amino group of alanine to α-ketoglutaric acid or oxalacetic acid by transamination [6] with the consequent formation of pyruvic acid. Direct deamination through the action of L-amino acid oxidase was claimed by Green and his associates [7]. Owing to the immense metabolic versatility of pyruvic acid, the implications of either of these reactions are numerous. The ready formation of pyruvic acid is also the simplest explanation for the role of alanine as the strongest carbohydrate-forming amino acid [8, 9, 10]. Since one metabolic path of pyruvic acid leads to the formation of acetic acid, the synthesis of acetate from alanine [11] is to be expected. Alanine is readily synthesized in the mammalian organism [13]. Kritzmann [12] believes that the synthesis in rat-liver slices occurs in presence of phosphate, ammonia, and bicarbonate in the following steps:

(1) carboxylation of pyruvic acid to oxalacetic acid;
(2) amination of oxalacetic acid to aspartic acid;
(3) transamination from aspartic acid to pyruvic acid, to yield alanine.

Alanine is abundant in most mammalian proteins and, when administered with the diet, takes part in rapid exchange reactions with protein [14, 15].

The comparative lack of complications in the metabolism of alanine has few counterparts with other protein constituents. Certain structural features contained in most of the other amino acids are responsible for the immense variety and complexity of their metabolism. The eight protein constituents which form the subject of the following chapters were selected because of their unorthodox metabolic behaviour. It is easy to see that the presence of a hydroxy group as in serine, that of sulphur as in cystine, and that of a methylated thiol group as in methionine, creates many metabolic possibilities which do not exist for a compound as simple as alanine. Furthermore, branched carbon chains, such as in valine and in the leucines, complicate their degradation. Glycine, the smallest protein constituent, and the only one without an asymmetric carbon atom, constitutes a metabolic class of its own.

In the light of these considerations an attempt to reduce the variety of biological reactions of the approximately twenty protein constituents to a few common metabolic principles is doomed to failure, and Kossel's view in 1912 seems true today: 'We obtain some idea of the possible variety in the combination and reactions of the protein constituents by recalling the fact that they are as numerous as the letters of the alphabet which are capable of expressing an infinite number of thoughts' [29].

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II

GLYCINE

Introduction

In the past glycine was considered a representative of the glucogenic protein constituents, heading the list of that group in most textbooks, and was believed to manifest a general behaviour characteristic for such an amino acid. As a result of investigations of various workers in recent years, this view has had to be considerably modified. On the grounds of the results described in subsequent sections, one is bound to arrive at the conclusion that the mode of utilization of glycine differs greatly from that of other glucogenic and urea-forming amino acids.

In neither the synthesis of glycogen, nor of urea does a stoichiometric relationship exist between glycine as the synthesizing agent and the products of reaction. Nor can the effect of glycine on such syntheses be ascertained without a considerable delay. Time-lags, it is true, are also found with other amino acids, e.g. with aspartic acid [28] and with alanine [31], but to a much lesser degree. Anti-ketogenic activity has often been claimed to manifest itself concurrently with gluconeogenesis, as was found to be the case with glycine in the experiments of Wick *et al.* [34*a*]. Both anti-ketogenic and glycogenic actions were observed only after a considerable time-lag in this instance, whereas with alanine or glucose both effects appear within a short time of administration [34]. These findings point to either a very slow general metabolism of glycine or to the complexity of its influence on nitrogen metabolism and carbohydrate synthesis. The first explanation can be dismissed on the grounds that glycine was found to act as an excellent 'donor' and 'acceptor' of dietary nitrogen [35, 35*a*]. The difference between glycine and a typical glucogenic amino acid, such as alanine, is particularly marked in experiments with isolated tissue preparations. Under such conditions neither carbohydrate synthesis nor urea formation can be demonstrated with glycine, while it can be readily shown with alanine (see below). Glycine is also exceptional in that of all amino acids administered to rats, it alone causes a marked and lasting increase in the amino-nitrogen content of muscle and liver (Luck [32]); further, the peak of glycogen deposition in the liver is only reached after a lag period of 14–16 hours [29, 34].

Incorporation of glycine into other molecules

Much evidence has been produced in recent years that *condensations* with, and *incorporations* into, other molecules are the most characteristic

metabolic features of glycine. In the majority of the reactions observed *the essential glycine structure remains intact*; further, by incorporation into another metabolite, glycine creates a new compound which in turn fulfils a new metabolic task. Such a behaviour is not usual for an amino acid, particularly with respect to its amino group, which in the case of glycine is relatively stable, whereas in most amino acids it is the first point of metabolic attack.

Many metabolic examples of this type are known, some of which are outlined below:

1. Condensation with aromatic compounds to form products of conjugation.
2. Condensation with arginine to form glycocyamine.
3. Incorporation of glycine into the uric acid molecule.
4. Incorporation of glycine into glutathione.
5. Incorporation of glycine into bile acids.
6. Incorporation of glycine into serine.
7. Incorporation of glycine into the haem molecule.

It was previously mentioned that in many cases the formation of metabolites from glycine is observed only after a lag of many hours. The 'overall oxidation' of glycine-carbon to CO_2, for instance, is, according to Weinhouse and Friedmann [280], greatly delayed and incomplete. The reactions listed above could possibly provide an explanation for this unusual behaviour, for it can be pictured that the degradation of the glycine molecule is preceded by its incorporation into other molecules in the 'framework' of which the degradation of the glycine moiety may eventually take place.

Carbohydrate synthesis *in vivo* can be envisaged to take place via the intermediary formation of serine, as will be described below. Urea synthesis from glycine will be delayed under conditions where glycine, before releasing its nitrogen, is, as a first metabolic step, built into other compounds.

The role of the 'carbon fragments' of glycine

While the synthesis of serine from glycine was being investigated, it came to light that glycine was not only incorporated into serine but was also degraded to one-carbon fragments with the formation of 'formate'. Following this observation, formate was found to act as precursor for the carbon of the labile methyl groups of choline and methionine. Moreover, formate condenses with another molecule of glycine to yield serine.

The realization of the degradation of glycine to simple one-carbon fragments makes it possible to assign to it the role of a general basic material, able to participate in the synthesis of larger molecules.

Furthermore, this behaviour of glycine could serve as an explanation for its characteristic stimulating effect on the metabolism of other compounds (see specific dynamic effect, p. 9). The degradation of its two-carbon chain to formic acid possibly, though not necessarily, passes through the reactive formaldehyde stage which lends itself to condensing reactions from which a synthesis of longer carbon chains may result. As will be seen in the following sections, labelled glycine-carbon was found in globin as well as in porphyrin; it was also found in the structure of glycogen and fatty acids [216]. Moreover, both carbon and nitrogen of glycine are found incorporated into protein chains and free amino acids.

Principal types of glycine reactions

Thus the utilization of glycine in the mammalian organism appears to be based on three principal metabolic reactions:

1. Glycine may be incorporated *as a whole* into other molecules: examples of such conjugating and incorporating reactions are listed above (see p. 7).
2. Glycine, after the loss of its carboxyl group but with its *intact carbon-nitrogen link*, is incorporated into other molecules, e.g. into the porphyrin molecule.
3. Glycine is degraded to *one-carbon fragments* such as 'formate', which in turn may take part in the resynthesis of a variety of other molecules.

Condensation of glycine with keto acids. The observation of Bach [14] that, on incubation of glycine with mixtures of tissue slices of different organs, much amino-N disappeared without a concurrent formation of urea, ammonia, or any other product of deamination investigated, and further that bisulphite-binding keto compounds (e.g. pyruvic acid) disappeared simultaneously with the amino-groups, led the author to suggest a condensation of glycine with these keto compounds. Such a condensation could have several consequences: when keto acids act as substrates for synthetic reactions, such as that of urea, glycine could act as inhibitor by preventing the keto acid from taking part in the synthesis. This was actually shown to be the case (Bach [224]). In other circumstances, where keto acids arise as products of reaction in oxidative processes (e.g. from the oxidation of lactic acid, aspartic acid, malic acid, etc.), a 'fixation' of the keto acids could cause a shift of the equilibrium within the enzyme systems concerned. Consequently, the reactions leading to the formation of such keto compounds would be accelerated. Such a view explains the results of analogous experiments of Green [225] in which cyanide, by cyanohydrin formation with oxalacetic acid, accelerated the oxidation of malic acid in tissue preparations.

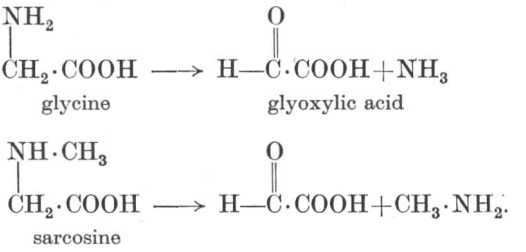
Specific dynamic action of glycine. The 'keto acid condensation hypothesis' was considered by Bach [14] to serve as a possible basis for the explanation of the high specific dynamic action of glycine which, with the exception of phenylalanine, is greater than that of any other amino acid investigated [27]. This explanation of the specific dynamic effect is compatible with the early view of Lusk [41] as well as with that of Borsook [40]. The former postulated a specific stimulation of the cells without the amino acids themselves undergoing oxidation. The latter defined the specific dynamic action as the increased energy production from the metabolism and excretion of the nitrogen as well as from the metabolism of the carbon moiety. But this definition is modified [40, p. 168] by the following limiting statement: 'It would be unjustifiable to conclude categorically that all the excess nitrogen (excreted) after the ingestion of protein is to be ascribed to the metabolism of the protein fed. Some of the ingested material . . . may be metabolized towards the end of the experimental period, and in so doing, may cause to be metabolized with it other nitrogenous substances, not ingested with the protein in question. . . .' The interpretation of experiments illustrating the specific dynamic action of glycine is difficult owing to various factors, e.g. the mode of administration, affecting the result [42*a*]. A full discussion of this complex problem will be found in the review by Borsook and others [40, 40*a*].

Deamination

Evidence for the deamination of glycine is based mainly upon the increase in urea excretion after glycine feeding or injection. The classical feeding experiments of Schultzen and Nencki [1] were soon confirmed by several later investigators [2, 3, 4]. Results from perfusion experiments on liver with glycine solutions were, however, not consistent. Salaskin was the first to report urea formation from liver perfusions [5], but his results were not fully confirmed by others [6, 7]. Nor did the use of the slice technique clarify the situation with regard to the deamination of glycine. Only a small amount of ammonia was produced from glycine after incubation with chopped kidney-tissue [8] and only a slight deamination of glycine was observed by Krebs [9] after incubation with kidney and liver slices. Later on the peculiar resistance of glycine to deamination became more evident [10–13], and in 1939 Bach showed [14] that no significant quantities of urea or ammonia were produced from glycine when incubated with slices or extracts of liver, kidney, or various other organs, nor when such organs were perfused with Ringer's solution containing glycine.

Yet Ratner *et al.* [15] isolated glycine oxidase (an enzyme of the flavoprotein type obtained from kidney and liver of some mammalian species), which catalyses the oxidative deamination of glycine to

glyoxylic acid and ammonia, as well as that of sarcosine to glyoxylic acid and methylamine.



Several points, however, indicate a limited physiological significance of this enzyme: the slow velocity of the glycine decomposition at physiological pH, the comparatively high Michaelis constant, and the toxicity of glyoxylic acid [16, 18]. The latter is slowly, but incompletely, oxidized in the body to oxalic acid which is also toxic [17, 18, 15, 280]. The role of oxalic acid in the organism is not clear [17, 19, 20]; later experiments of Weinhouse and Friedmann [280] reveal its remarkable metabolic inertness.

If glycine were deaminated to glyoxylic acid, its administration would be expected to cause an increased formation of oxalic acid in the organism (see above). For this there is, however, no evidence. (For references see [280].) Neither glyoxylic acid, nor glycollic acid is glucogenic [16], so that glycogen formation from glycine *in vivo* cannot easily be pictured to occur with the oxidation of glycine to glyoxylic acid as the first step. On the other hand, the very slight deamination of glycine observed in tissue slices and tissue-extract experiments [8–9] may well be the result of the action of glycine oxidase or of other deaminases present in Ratner's preparation [15].

At any rate, the above observations make it difficult to consider the deamination of glycine to glyoxylic acid a principal mechanism in the intact animal; several other observations point to the comparative stability of the amino group in the glycine molecule.

From experiments on the synthesis of haem from glycine [116] Shemin and Rittenberg reported that the administration of nitrogen-labelled ammonium citrate caused no significant increase of the isotope concentration in the haem of the rat. This indicated that glycine was not deaminated prior to its conversion into the haem molecule. Further, both Radin *et al.* [254] and Muir and Neuberger [250] emphasize that during the synthesis of haem from glycine no reversible deamination could have taken place; Shemin [83] arrived at the same conclusion.

Nor is, as pointed out before, the position of glycine firmly established with regard to urea synthesis. In experiments with tissue preparations with added glycine, neither ammonia nor urea formation can be ascer-