Biochemistry of Bacterial Growth

EDITED BY

JOEL MANDELSTAM
KENNETH MCQUILLEN

IAN DAWES

THIRD EDITION

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JOEL MANDELSTAM
Microbiology Unit
Department of Biochemistry
University of Oxford

KENNETH McQUILLEN
Sub-Department of Chemical Microbiology
Department of Biochemistry
University of Cambridge

IAN DAWES
Department of Microbiology
University of Edinburgh

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Osney Mead, Oxford, OX2 0EL
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9 Forrest Road, Edinburgh, EH1 2QH
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Contributors

S. BAUMBERG, *Department of Genetics, University of Leeds*

E. A. DAWES, *Department of Biochemistry, University of Hull*

IAN W. DAWES, *Department of Microbiology, School of Agriculture, University of Edinburgh*

D. KERRIDGE, *Sub-Department of Chemical Microbiology, Department of Biochemistry, University of Cambridge*

P. J. LARGE, *Department of Biochemistry, University of Hull*

KENNETH MCQUILLEN, *Sub-Department of Chemical Microbiology, Department of Biochemistry, University of Cambridge*

J. MANDELSTAM, *Microbiology Unit, Department of Biochemistry, University of Oxford*

R. H. PRITCHARD, *Department of Genetics, University of Leicester*

P. E. REYNOLDS, *Sub-Department of Chemical Microbiology, Department of Biochemistry, University of Cambridge*

JOHN G. SCAIFE, *Department of Molecular Biology, University of Edinburgh*

D. W. TEMPEST, *Laboratorium voor Microbiologie, Universiteit van Amsterdam*

MICHAEL J. WARING, *Department of Pharmacology, University of Cambridge*

Preface to Third Edition

Because it is conventional to reprint prefaces from all previous editions there is little point in reiterating the aims set out in the First Edition (since they remain the same) or in attempting to rebut the opinions of the few reviewers who misunderstood them. We do, however, again express our thanks to colleagues who have made many helpful suggestions and have provided valuable material for illustrations.

Since the second edition was published, knowledge of the biochemistry of bacterial growth has increased much as bacteria themselves increase in a favourable environment. This new edition reflects these changes, in particular, with new presentations of the subjects of Growth, Genetics, and Differentiation. More detailed treatment is given to the division of individual cells and to the replication of chromosomes, while growth of cell populations in continuous cultures has received much more attention. The chapter on Genetics has been re-written to take account of major advances in the biochemistry of DNA and of the application of techniques for genetic manipulation both *in vivo* and *in vitro*. Previously, the treatment of differentiation was restricted to sporulation and germination. The chapter now deals with a much wider range of cellular structures and developmental processes, and the emphasis is on what can be explained in molecular terms. The form of the remaining chapters has been retained but the content has been up-dated where necessary.

In both of the earlier editions a very brief summary was used to introduce a general development of the whole subject in about fifty pages and this was followed by detailed treatment of the same material expanded about tenfold. This presentation seems to have been found useful both by teachers and by students. We have retained it.

JOEL MANDELSTAM
KENNETH MCQUILLEN
IAN W. DAWES

Preface to First Edition

This book is not intended to be a comprehensive textbook on the biochemistry of bacteria. It has been written in the belief that the advances in biochemistry in the last ten years provide a basis for a fairly comprehensive description of bacterial life in biochemical terms, and that such a view of the bacterial cell can with advantage be presented to beginners. We also believe that the most recent concepts are as readily intelligible as the older and more basic ideas. For this reason we have, for example, thought it just as easy, and much more interesting, for the student to learn the modern view of replication of the bacterial chromosome before he learns the structural formulae of the nucleotides. Similarly we have presented the 'coding problem' in protein synthesis before introducing the chemical structures of the twenty common amino acids. As far as possible this method of approach has been followed throughout.

We have also attempted to build up from the start a coherent picture. Too often in the teaching of biochemistry the student is taken through one detailed aspect of the subject after another. Only at the end does he have all the information which will allow him to construct some sort of integrated picture. By this time his mind may be so clogged with details that the process of fitting them together is needlessly difficult. Our method of presentation will, we hope, avoid this danger and it has resulted in a book written in three parts. The introduction is a summary of the book in a few pages and it is based upon a very general account of what a bacterial cell does during growth. This is followed in Part I by a somewhat more detailed description of the same material; it presupposes very little knowledge apart from some basic chemistry. If we have been successful in our exposition, the student should, at this stage, have a clear picture—still in very general terms—of a bacterial cell as an integrated biochemical system. The detailed biochemistry will be found in Part II, the third and largest part of the book. We realize that the subject may seem to be so oversimplified by this treatment that the impression is given that everything in bacterial life is now explicable in biochemical terms apart from a few minor gaps. We have tried to avoid this by stressing, particularly in the conclusion, those phenomena which are as yet not reducible to biochemistry, and which are likely to be the growing points of the subject.

Finally, we have avoided the historical approach which, while it may be the most scholarly, is for the reader the dullest. It can, furthermore, reasonably be argued that it usually fails in its object. The significance of early discoveries and controversies is best appreciated by those who already know the subject fairly well, and not by beginners: it is for the latter that the book has been written.

In our attempt to co-ordinate the chapters of this book we have inflicted our views and prejudices upon the contributors to a considerable extent. This was particularly true during the preparation of Part I which has now been written and re-written so many times that it is impossible to attribute individual authorship to the sections. We are grateful to the authors for their tolerance and patience. We are also most appreciative of the willing help and co-operation of everyone at Blackwell Scientific Publications who was concerned with this book, in particular Mr Per Saugman, Mr John Robson and Miss Yvonne Prince.

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Introduction
Abstract of the Book

This chapter is a highly condensed introduction to the biochemical events that underlie bacterial growth. It is, at the same time, intended to be a summary description of the contents of the rest of the book.

For a model system it will be convenient to choose an unspecified bacterium that can grow in a medium with glucose as the carbon source. Its nitrogen requirements are satisfied by ammonium ions and its sulphur requirements by sulphate. Magnesium and phosphate are essential and it needs trace amounts of other metals (e.g. iron). It can be considered as a 'generalized bacterial cell' and we shall attribute to it a mixture of the properties found in several different kinds of bacteria. It should be regarded as an abstraction in much the same way as the 'average man'. Real bacteria will be considered in the main section of the book and some of the ways in which they differ from the model will become apparent.

The organism is represented schematically in Figure 1. It is rod-shaped and has a rigid outer wall that maintains and supports the membrane that it encloses. The wall is made of a polymer substance, the peptidoglycan, and the membrane contains proteins and lipids. These coats surround the cytoplasm, which consists mainly of polymers: deoxyribonucleic acid (DNA); ribonucleic acid (RNA); proteins and polysaccharides. In terms of dry weight the polymers account for about 90% of the cell (see Figure 2). The remaining 10% of the cell is made up of a large variety of small molecules: amino acids, nucleotides, growth factors, fats. Although these constitute so small a fraction of the cell mass, they are metabolically of great importance.

Not only do the macromolecules make up the bulk of the bacterial cell, they also give it the characteristics that distinguish it from all other types of bacteria. The small molecules, on the other hand, are common to all types of bacteria and, indeed, to other forms of life.

When the organism is in a suitable environment or growth medium, more of all these materials is produced and in due course the cell divides into two daughter cells indistinguishable from one another and from their parent. The subject of this book is a description of the way in which the simple organic and inorganic constituents of the medium are transformed into new cell material with its enormous diversity of molecular species.

Classification of biochemical reactions

The number of chemical reactions involved in growth is unknown but is probably of the order of a thousand. Of these a few may occur spontaneously but the vast majority have to be catalysed by specific

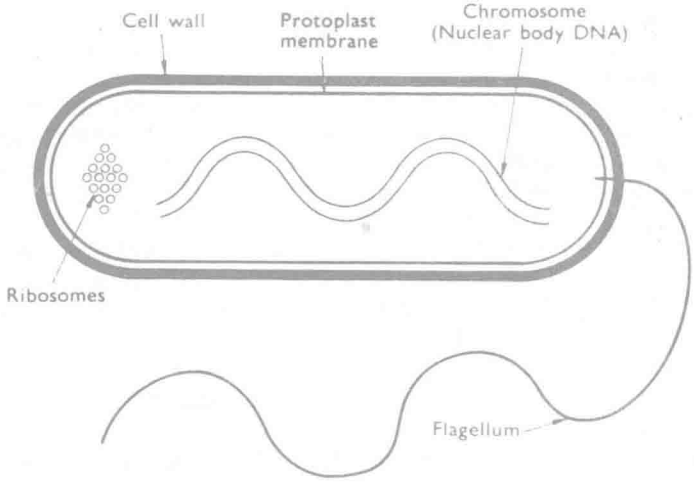


Figure 1. Diagrammatic representation of a bacterial cell.

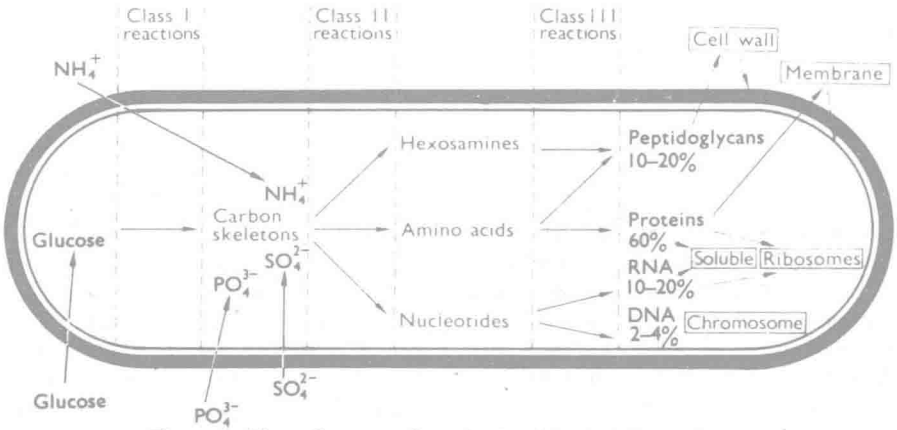


Figure 2. Flow diagram of synthesis of bacterial constituents.

proteins, the *enzymes*. Each of these catalyses a specific reaction such as the addition or removal of water, or hydrogen, or 1-C residues, or amino groups, etc. (see Appendix B, p. 405). For our purposes enzyme reactions can be grouped into three classes.

Class I (degradative reactions)
There is first a complex of enzymes which degrades glucose to smaller aliphatic carbon compounds. This class will be called degradative enzymes. The net process is exergonic, i.e. produces energy. It also results in the supply of carbon skeletons for synthetic reactions.

Class II (biosynthetic reactions)
From these carbon skeletons a further series of enzymes catalyses the formation of the small molecules which are the basic components of the macromolecules. Many of these intermediates (amino acids, nucleotides, hexosamines) contain nitrogen which is derived from the NH_4^+. Some contain sulphur which comes from SO_4^{2-}. At the same time some small molecules (vitamins, co-factors) are synthesized but are not incorporated into macromolecules; rather, they are needed for the proper functioning of the enzymes. The enzymes producing all these substances will be called biosynthetic, Class II. As a group they largely require energy and are therefore endergonic. The energy is produced by the Class I reactions.

Class III (biosynthetic reactions)
A further series of enzymes then converts the basic small molecules into macromolecules. When enough of these have been synthesized the cell divides. Since the distinctive character of the cell is determined by its macromolecules, much of this book will be concerned with the mechanisms by which these complicated structures are reproduced so exactly.

The genetic information for copying the cell is carried in its DNA which is the 'blueprint' for the whole cell, that is, *all* the information determining what the biochemical machinery shall be, and how it will be put together, is encoded in the DNA. When the cell divides each of the daughter cells must, apart from anything else, receive a complete copy of the 'blueprint'. It is essential that the DNA molecules should be copied correctly at every division because, as in any highly organized system, a random error will almost certainly be damaging. It is only *very* rarely that such an error will be advantageous. We have thus two separate problems to consider. Firstly, how an exact copy of the DNA is made and then, when this has happened, how the information in it is translated into the other types of molecules.

The DNA molecule is a very long polymer made up of four kinds of nucleotide joined through their phosphates. All four contain the sugar *deoxy*ribose and are represented by dA, dG, dC, and dT because of the four different bases (see Figure 3, p. 12 for their chemical structures). The properties of these nucleotides are such that dA and dT have an affinity for each other and so have dG and dC. Thus if we have a chain as follows:

—dA—dA—dG—dT—dC—dG—

then free nucleotides will be assembled into a

sequence in accordance with their pairing properties, thus:

—dA—dA—dG—dT—dC—dG— Original chain

—dT—dT—dC—dA—dG—dC— Complementary sequence

Cells possess an enzyme (DNA polymerase) that links these nucleotides covalently to give a polymer that will be exactly complementary to the original template. This complementary chain can itself be copied, again in accordance with the pairing properties of the nucleotides, thus giving a strand identical with the original chain:

—dT—dT—dC—dA—dG—dC— Complementary chain

—dA—dA—dG—dT—dC—dG— Identical copy of original chain

The complementary chain can be regarded as the biochemical equivalent of a photographic negative. This explains in an over-simplified way the principle of DNA replication. In fact, the DNA exists as a double-stranded structure that is unravelled during the copying process (see p. 26 and Chapter 5).

So far, then, we have accounted for the formation of a DNA molecule containing all the necessary information for the hundreds of enzymes which will catalyse the three classes of reactions we have described. Now, these reactions are responsible for all the materials that the cell contains and for all the biochemical reactions it can carry out. Our problem is thus reduced essentially to that of understanding how the information in the DNA is translated into that of the enzyme proteins. The information for any particular species of protein is carried in a stretch of DNA which may contain more than 1000 nucleotides and which is known as the *structural gene* for that protein. The enzymes and other proteins consist of chains containing twenty kinds of amino acid. The number of amino acid residues and their order are different for each kind of protein. The problem was to find the way in which the four types of nucleotide in a stretch of DNA specify the 20 types of amino acid in a protein. This was formally analogous to finding out how the two-letter system (dots and dashes) of Morse code could be translated into the ordinary alphabet of 26 letters and it was therefore referred to as the coding problem.

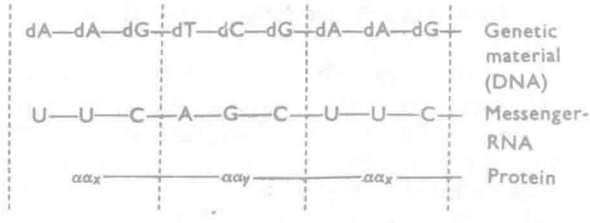
The translation of DNA into protein occurs in a number of steps. First, the informational content of the structural gene is transferred to a strip of RNA known as the *messenger-RNA*. RNA is also a polymer consisting of four kinds of nucleotide, but in this nucleic acid they all contain ribose instead of deoxyribose and they have U instead of T. They are represented by A, G, C and U. However, pairing can occur between these nucleotides and those of DNA. In the presence of the appropriate enzyme (RNA polymerase) and a DNA template, the four ribonucleotides are polymerized into a complementary copy of the DNA strand:

dA—dA—dG—dT—dC—dG— DNA template

U—U—C—A—G—C— Complementary RNA

Thus DNA fulfills *two* separate template functions. The first, mediated by DNA polymerase, is to serve as a template for its own replication: the second is to act as a template for the production of the complementary messenger-RNA.

The messenger-RNA acts as a template on which amino acid residues are assembled in correct sequence before being linked together. The solution of the coding problem is that the code is triplet, i.e. that a sequence of three nucleotides codes for each amino acid:



Here the DNA triplet, dA—dA—dG is transcribed into the complementary messenger-RNA triplet, U—U—C which is translated as an amino acid ($\alpha\alpha_x$), etc. Each succeeding triplet causes the insertion of the next appropriate amino acid until the protein is complete. The whole chain might easily contain 300 amino acid residues. The assembly of proteins is, however, more complex than this description suggests and involves the participation of other types of molecules.

The remaining macromolecules to be considered are the polysaccharides and peptidoglycans. The biosynthesis of these is generally simpler than that of the proteins. Some, like glycogen, are polymers containing only one type of sugar residue. Theoretically a single enzyme could string together a chain of such residues to form a polymer. In fact most polysaccharides are more complex, but even so only a few enzymes are required for the synthesis of any one of them. The peptidoglycans are somewhat similar, containing two kinds of amino sugar occurring in regular alternation. They also have short side chains of amino acids but their assembly is achieved by a fairly small number of enzymes.

The number and types of small molecules that a cell can make and degrade are determined by its content of enzymes.

Genetics

So far all the processes we have outlined may be considered as taking place in a single bacterium. They involve the conveyance of information in the DNA to the rest of the cell material. However, information can also be conveyed *inter*-cellularly. In bacteria this can be effected in one of three ways: (a) by a mating process; (b) by transformation—the direct uptake by one cell of free DNA liberated from another cell, by lysis or otherwise; (c) by transduction. Here an infective virus particle during its formation picks up some of the DNA of the host cell and then transfers it to the next bacterial cell it infects.

Growth and the regulation of biosynthesis

We can now summarize the events that take place when some viable cells are placed into growth medium. Some of their enzymes degrade glucose, some synthesize basic molecules and yet others assemble macromolecules including more of all the enzymes. With more of all these catalysts thus available, the same processes will continue but at an accelerated rate, giving yet more enzyme, and more cells. The rate of synthesis is thus proportional to the amount of cell material present and this leads to an exponential rate of growth that continues until something in the environment becomes limiting. When this happens some types of bacteria simply cease to grow but others form spores which are heat-resistant and can lie in a dormant state for many years. Subsequently, if the environment becomes favourable, these can germinate and begin to grow again. Sporulation and germination are among the most primitive forms of cell differentiation and are consequently of considerable interest.

Returning to the actively growing cell, let us consider its internal economy. It has to produce 20 types of amino acids for its proteins, four types of nucleotides containing deoxyribose for DNA, four more containing ribose for RNA and also a variety of co-factors and lipids. The synthesis of any one of these substances may easily involve ten or more specifically catalysed steps carried out by enzymes of Class II. In addition there is a considerable number of intermediates produced from glucose by the Class I enzymes.

For efficient growth all the basic materials and all the macromolecules derived from them have to be produced in the correct proportions. Under natural conditions, bacteria are often in competition for a limited amount of nutrient. The consequence is that a very efficient regulation mechanism has evolved. Since virtually all metabolic steps are enzymically catalysed, in considering metabolic regulation, we have really to consider regulation of enzymic function. There are two ways in which this can occur. One is by alteration of the *amount* of any particular enzyme, the other is by alteration of the *rate* at which it functions. Both types of regulation are found in the bacterial cell and their combined effect ensures that the cell is geared to get the maximum yield of protoplasm from its environment and to do so in the minimum time.

原书缺页

原书缺页

Part I
Section 1
The Bacterial Cell: Major Structures

Bacterial cells occur in a variety of shapes and sizes depending on the kind of organism and on the way in which it has been grown, but for many purposes it is possible to disregard these variations and to consider the common properties of the 'generalized bacterial cell'. Thus, although bacteria may be spherical or curved or spiral, the majority are rod-shaped and are about 1 μm wide and 2 μm in length (1 μm = 0·001 mm). A single bacterial cell may thus have a volume of 10^{-12} ml and contain $2{\cdot}5 \times 10^{-13}$ g of dry matter (equivalent to a relative molecular mass, M_r, of $1{\cdot}5 \times 10^{11}$). But this bacterium is not just an undifferentiated blob of 'protoplasm'. It is a highly organized structure with organelles corresponding in function to many of those found in higher organisms. The hereditary material (DNA) is embedded in the *cytoplasm* which, surrounded by the *cell membrane*, is called the *protoplast*. Outside this lies the *cell wall*.

Cell walls and membranes

The wall is fairly rigid and gives shape and protection to the cell. It amounts to about 10% of the weight of the entire cell. Always there is present in it peptidoglycan, and this seems to be what makes the wall rigid. The peptidoglycans are made of chains of amino sugars, *N*-acetylglucosamine alternating with *N*-acetylmuramic acid. Short peptides are linked to the muramic acid residues and separate chains are joined by these peptides to form the relatively thick structure needed for a wall (Figure 1).

The amino sugar, muramic acid, has not been found in any biological polymers other than the peptidoglycans of the cell walls of bacteria and the closely related blue-green algae. The peptides are also interesting in that they contain unusual amino acids. Besides L-alanine they contain D-alanine and D-glutamic acid, the so-called 'unnatural' isomers which are not present in proteins. Most species also contain diaminopimelic acid which, like muramic acid, is restricted in nature to peptidoglycans.

Other polymers which may occur in cell walls include teichoic acids, lipopolysaccharides and lipoproteins (see Chapter 1).

In species in which the wall is mainly peptidoglycan it is sometimes possible to digest it away with an enzyme called *lysozyme* which occurs in secretions such as tears and sweat and also in white of egg. Enzymic digestion of the wall releases the protoplast, but this is likely to burst unless given some osmotic protection. This is because the concentration of intra-cellular solutes exerts an osmotic pressure equivalent to 5–25 atmospheres. A solution of sucrose (10–20% w/v) will usually prevent this lysis of protoplasts. No matter what the shape of the cell, the naked protoplast on release from the cell wall assumes a spherical shape. It is bounded by a very delicate membrane called variously the *plasma membrane*, the *protoplast membrane*, or the *cytoplasmic membrane*. The structure consists predominantly of protein and lipid, as do all biological membranes, and it has a thickness of about 8 nm—this is dictated by the dimensions of the molecules of which it is composed. The membrane is the main permeability barrier of the cell, since the wall is freely penetrated by most molecules except very large ones. Some substances pass into and out of cells by passive diffusion but many are transported by highly specific systems which require energy and are located in the cell membrane. The name *permease* or sometimes *translocase* is given to such a system. As far as passive diffusion is concerned, smaller molecules and substances of high lipid solubility penetrate membranes more easily than do larger molecules and polar substances. For instance, C_4 sugars and some C_5 sugars may pass freely but other C_5 and all C_6 sugars (including glucose) may fail to penetrate by passive diffusion except very slowly. Bacterial cells are also generally impermeable to small cations and to inorganic phosphate ions. These non-penetrating substances have to be actively transported into the organisms.

Proteins and nucleic acids

Three classes of polymers are found in all bacteria. These are the proteins, and the two kinds of nucleic acids. Viruses, on the other hand, contain protein and *either* DNA *or* RNA.

Structure of proteins

The proteins perform various functions, some catalytic and some structural, and it is this class of sub-

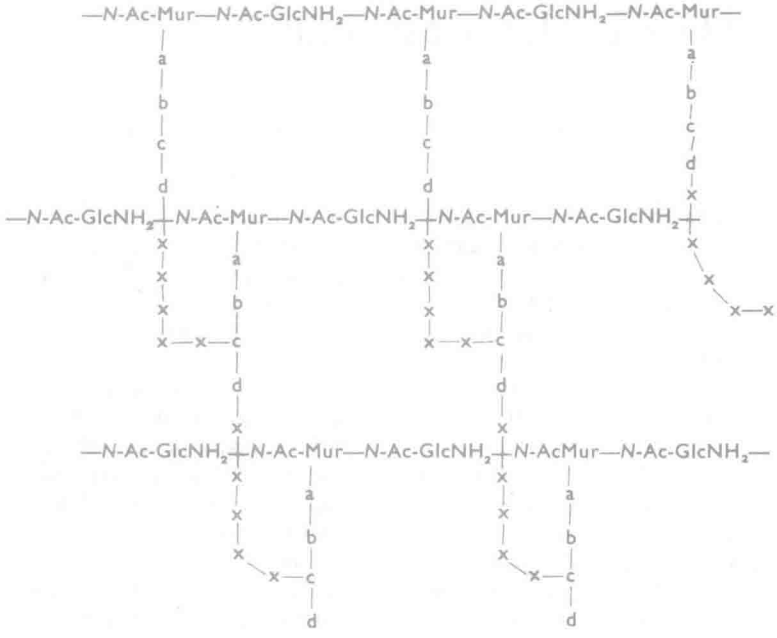


Figure 1. Structure of cell wall peptidoglycan.

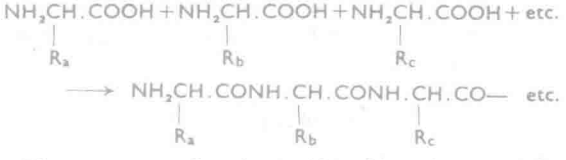
Alternating amino sugars (*N*-acetyl-glucosamine and *N*-acetyl-muramic acid) form a backbone with a peptide (a-b-c-d) attached to a muramic acid residue. These peptides may be linked through further amino acids (x-x-x-)

stance which determines the identity of an organism, that is, its membership of a particular species. About half of the dry weight of a bacterium consists of protein but there may be more than 1000 different species of protein in any one cell. The size of these polymers ranges in relative molecular mass from a few thousands to millions but each has a definite, precise composition.

The constituents of proteins are the amino acids, having the general formula:

see Figure 2

They occur with various frequencies and in various orders in the proteins. A simple protein may consist of a single polypeptide chain formed by the condensation of amino acids:



The sequence of amino acid residues in a protein or polypeptide is called its *primary structure*. Physical studies have shown that the chains do not exist in a straight, extended form but often coil into a spiral with $3\frac{2}{3}$ amino acid residues per turn, that is, eleven for each three turns of the helix. This α-helical structure is called the *secondary structure* and it is stabilized principally by so-called hydrogen bonds (H-bonds) between  residues in the chain.

Many proteins consist not of one but of several polypeptide chains joined together, and this is frequently achieved through —S—S— bridges. The amino acid cysteine possesses an —SH group in its side-chain and residues of cysteine in two chains may couple together thus: