MICROBIOLOGY AND IMMUNOLOGY

for the health team

Peter R. Stewart
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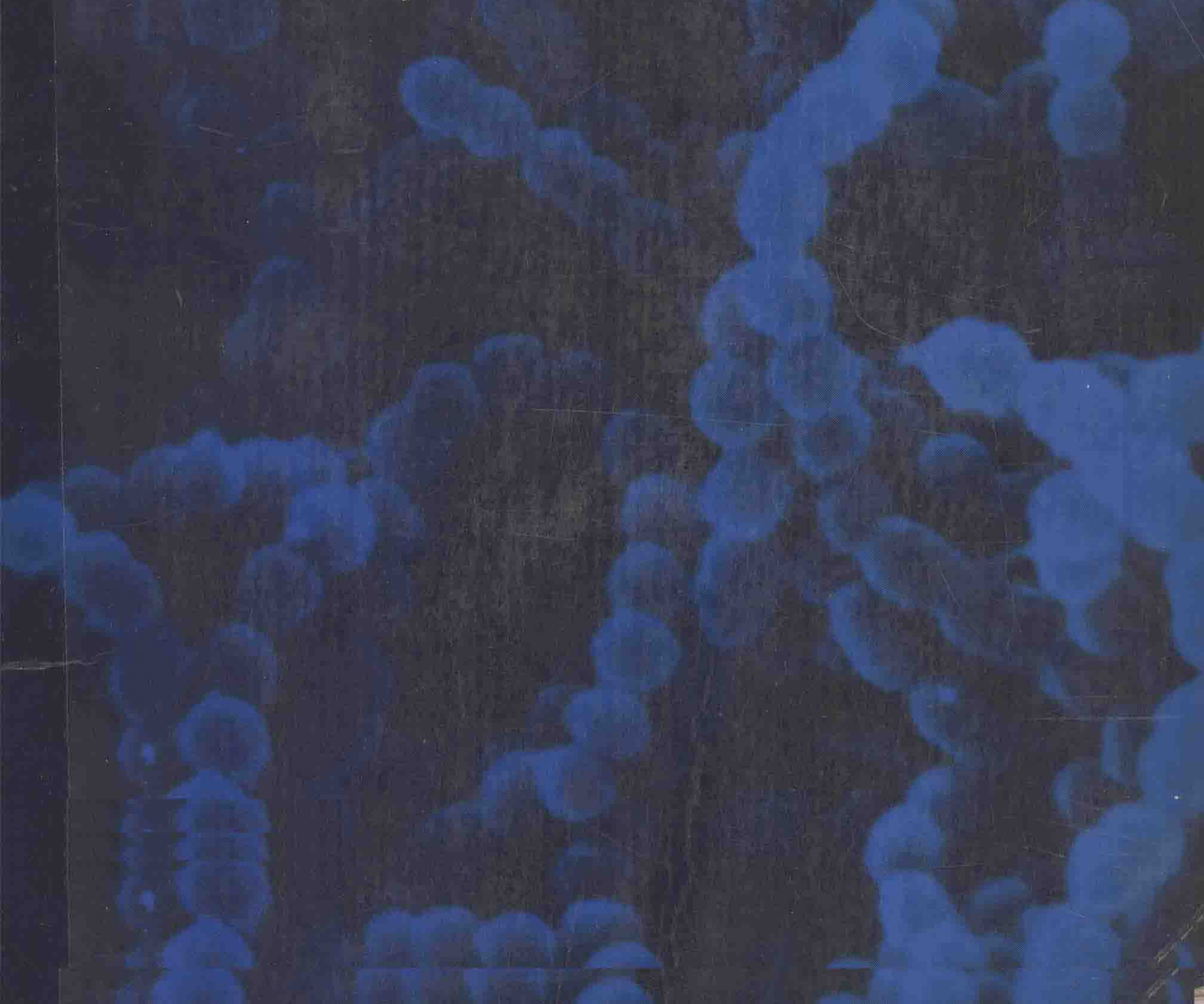

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Part 1

Background to Microbiology

Chapter 1

The nature of microorganisms

Microbiology is the scientific study of microorganisms, and is a branch of biology which parallels zoology, botany, medicine and human biology. It is distinguished from these other areas of biology by its subject — microbes or microorganisms. The procedures and techniques by which microorganisms are studied are often different from those used to study plants, animals and ourselves. This is because microbes are:

- very small, and therefore can be seen only with a microscope
- frequently studied as populations containing very large numbers of individuals.

To understand how microbes differ from plants, animals and ourselves, the concept of organism must be kept in mind. Organism, in modern biology, means a complete living unit capable of an essentially independent or autonomous existence. In the case of microorganisms, the organism is one or a few cells; for macroorganisms, such as animals and plants, the organism is many thousands or millions of cells.

Microorganism thus means a microscopic, autonomous, living entity; and microbiology is the study of such organisms. It is the remarkable capacity for independence and flexibility built into these minute, individual specks of life, with a basic existence quite separate from other living cells, which represents the wonder and fascination of the microbial way of life.

The scope of microbiology

The diversity of microorganisms is comparable to that seen among macroorganisms, such as plants and animals. Microorganisms may be subdivided as follows:

- protozoa
- algae
- fungi and yeasts
- bacteria
- viruses
- plasmids

approximately on a scale of decreasing organisational complexity. Viruses and plasmids are included in this list, though, as we shall see later, they are not in fact cellular organisms and cannot exist independently of a living host cell.

The study of protozoa (protozoology) has close links with zoology, since the cells of these organisms are similar to animal cells. The study of fungi and yeasts (mycology) and of the microscopic algae (phycology), on the other hand, has in the past often been related to botany — algae, because they are photosynthetic organisms, and fungi and yeasts because they have thick cell walls, similar in some ways to the cellulose walls surrounding plant cells. It is thus difficult to distinguish precisely where microbiology begins and ends when considering it in relation to the study of plants and animals. The scientific study of viruses (virology) has special features because of the non-cellular properties of these entities, which seem to be partly living organisms and partly inert, as we shall see. Plasmids are similar to viruses in many ways, but are even simpler chemically and structurally.

Bacteriology has been used as a synonym for microbiology, but the word should be reserved to indicate the study of bacteria. Finally, in this list of 'ologies' related to microbiology, immunology should be included. In a classical sense this is the study of the response of animals or of the human body to invasion by pathogenic microbes. It focuses on those persisting changes in the blood and other tissues which are an integral part of immunity to disease. More recently, immunologists have become concerned with a wider range of aspects of an organism's response to other organisms or to substances which are 'not-self': transplantation, cancer and cell differentiation, the response of the body's immune system to itself (autoimmunity), and genetic aspects of many of these immunological phenomena.

Aspects of all these facets of microbiology and immunology are included in the following chapters. Unavoidably, important matters have had to be left out. Among these are the industrial uses of microorganisms in the production, for example, of antibiotics and drugs, the processing of raw agricultural materials into such products as cheese, bread and wine, or the growing interest in microbes for waste disposal, energy generation, and protein production. For the reader interested in these topics there are excellent summaries in the texts listed at the end of this chapter.

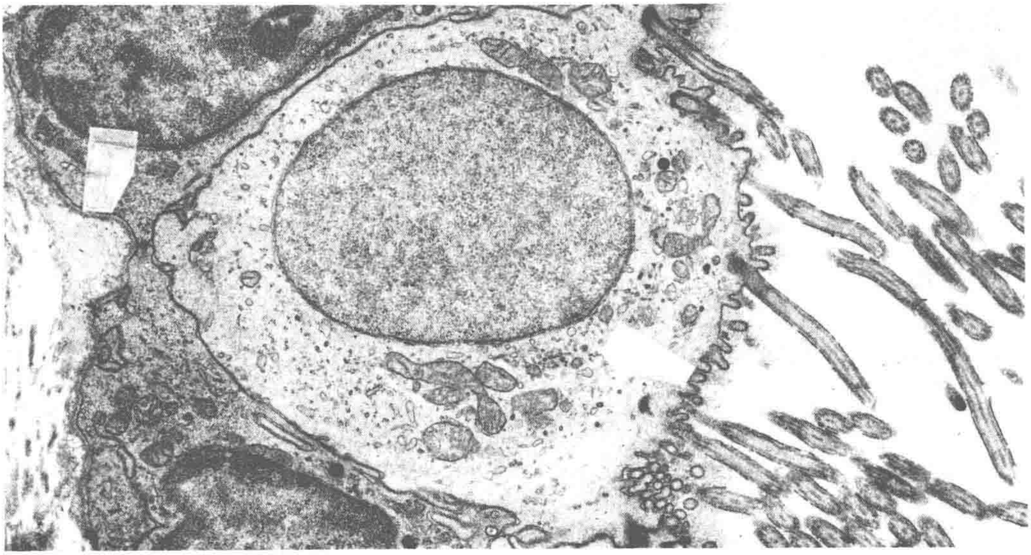
The cell as the basic unit of life

There is no structure or aggregate of biological molecules smaller than a cell that can exist and multiply independently. By life we mean the capacity to transform energy and to assemble molecules to reproduce the original unit. Only cells, and aggregates of cells, can do this, though viruses and plasmids present a particular alternative which we shall consider in some detail later.

The concept of macroscopic organisms being built up from very large numbers of individual living cells is an old one. Within an animal or plant, large numbers of similar types of cells are grouped together to form organs or tissues (e.g. liver, muscle, skin) which have specialised structural or physiological and biochemical functions. Each cell is dependent in some way on other cells either nearby, or remote, since the life of the organism as a whole is dependent on the correct and integrated functioning of the various tissues and thus of the cells which compose it. Plant and animal cells are not individually self-sufficient, and can survive and reproduce in nature only when they are part of the whole organism.

In dealing with microorganisms, the concept of the cell as the basic unit of life remains, except that, as previously mentioned, one cell (or a few cells at most) is the organism, and hence each cell must possess the diversity of functions that in macroorganisms is encompassed by large numbers and types of specialised cells. The microbe is at one and the same time the stomach cell, lung cell, kidney cell, reproductive cell, and so on.

Moreover, because many microbes live in an environment which may continually be changing in temperature, acidity, chemical composition and so forth, microbes must also



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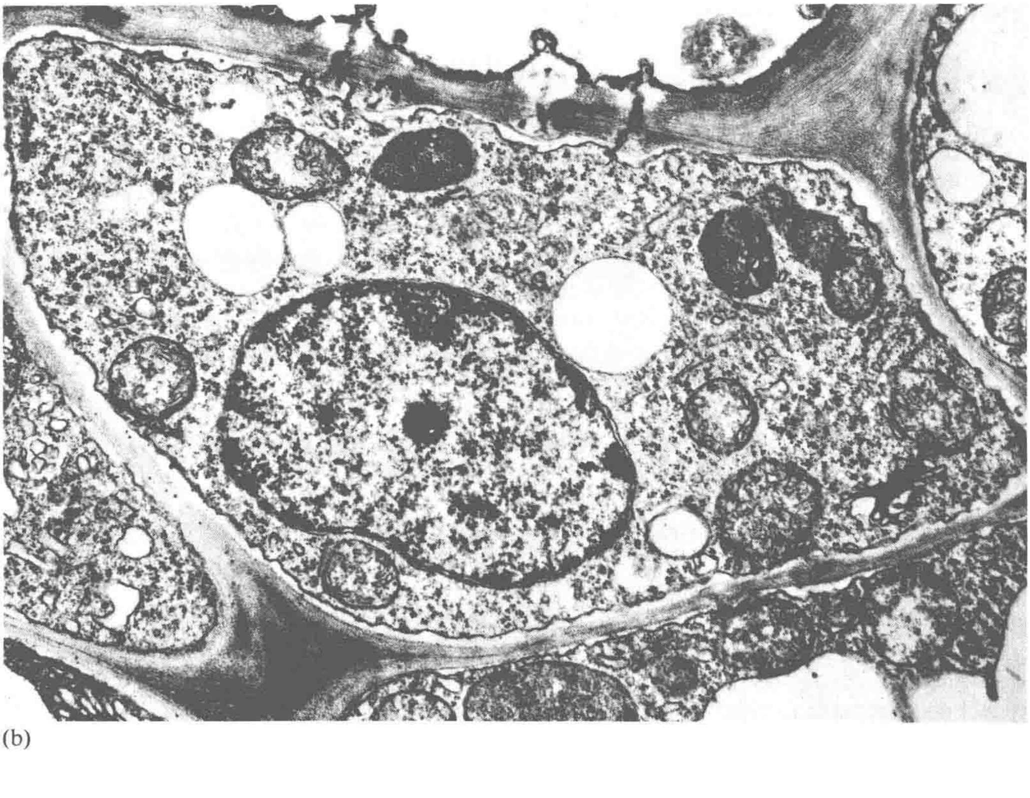

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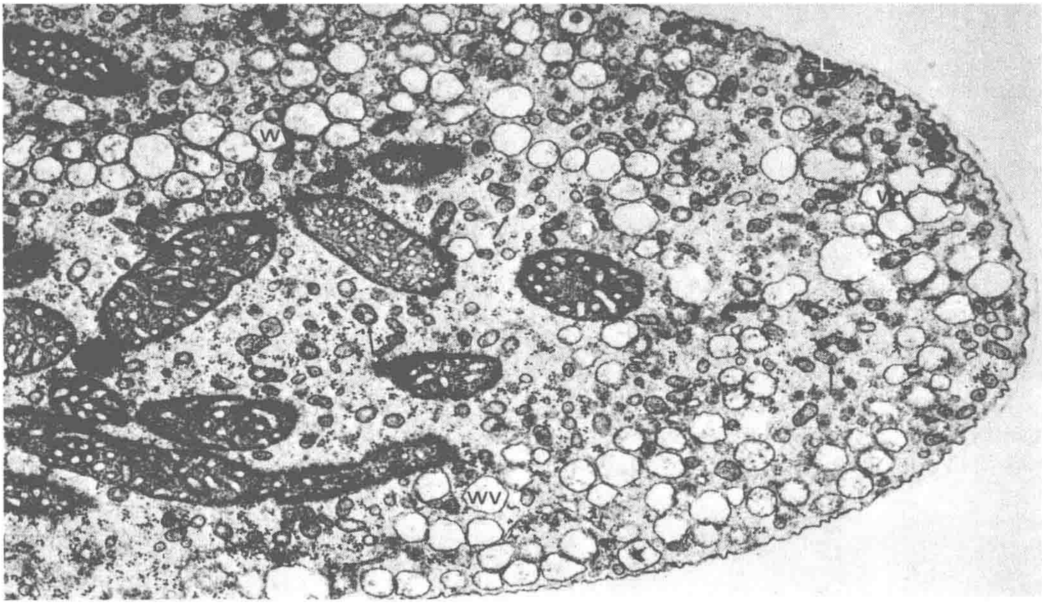

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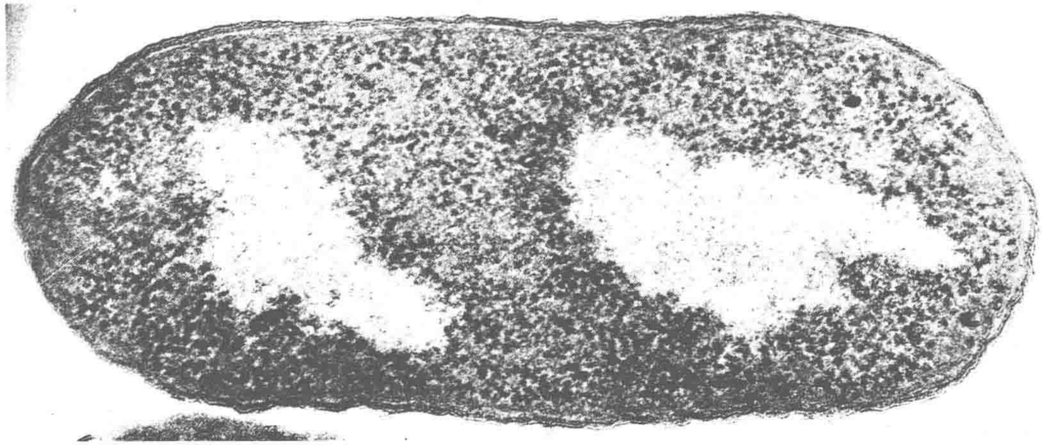

(d)

Figure 1.1 *Representative cell types: animal (a); plant (b); fungus (c); bacterium (d)*

The bacterial cell has a lesser degree of complexity of internal structure; the animal cell does not have a surrounding cell wall; and animal and plant cells are part of a multicellular tissue, each cell in intimate contact with adjacent cells, whereas the microorganisms illustrated (fungus, bacterium) are individual cells in immediate contact with their physical environment.

The magnification of the electron micrographs are not the same. The animal and plant cells are 10–12 μm across, the fungal filament thickness is about 5 μm, and the length of the bacterium is approximately 2 μm.

Sources: (a) K. R. Porter and M. A. Bonneville, *Fine Structure of Cells and Tissues*, Lea and Febiger, Philadelphia, 1973

(b) B. E. S. Gunning and M. W. Steer, *Plant Cell Biology — An Ultrastructural Approach*, Edward Arnold, London, 1975

(c) A. Beckett, I. B. Heath and D. J. McLaughlin, *An Atlas of Fungal Ultrastructure*, Longman, London, 1974

(d) M. A. Tribe, M. R. Eraut and R. K. Snook, *Electron Microscopy and Cell Structure*, Cambridge University Press, Cambridge, 1975 be adaptable to these changes. Cells from macroscopic organisms are much less flexible and adaptive, since they are nurtured within the internal environment of the organisms and therefore buffered against external environmental change.

The cell, as the unit of life, exemplifies the paradox of diversity yet unity which we know from observation to be true of macroscopic life about us. Shape, size, mobility and function are as different among individual cells as they are among macroscopic plants and animals (Figure 1.1). But, at the root of it all, the biochemical basis of growth, movement, reproduction, and organisation in each and every cell is extraordinarily similar. This biochemical similarity will be considered again later.

Finally, it should be said that the concept of the cell as the basic unit of life, like all useful generalisations, has important exceptions. These occur primarily in the microbial world in one of three predominant forms:

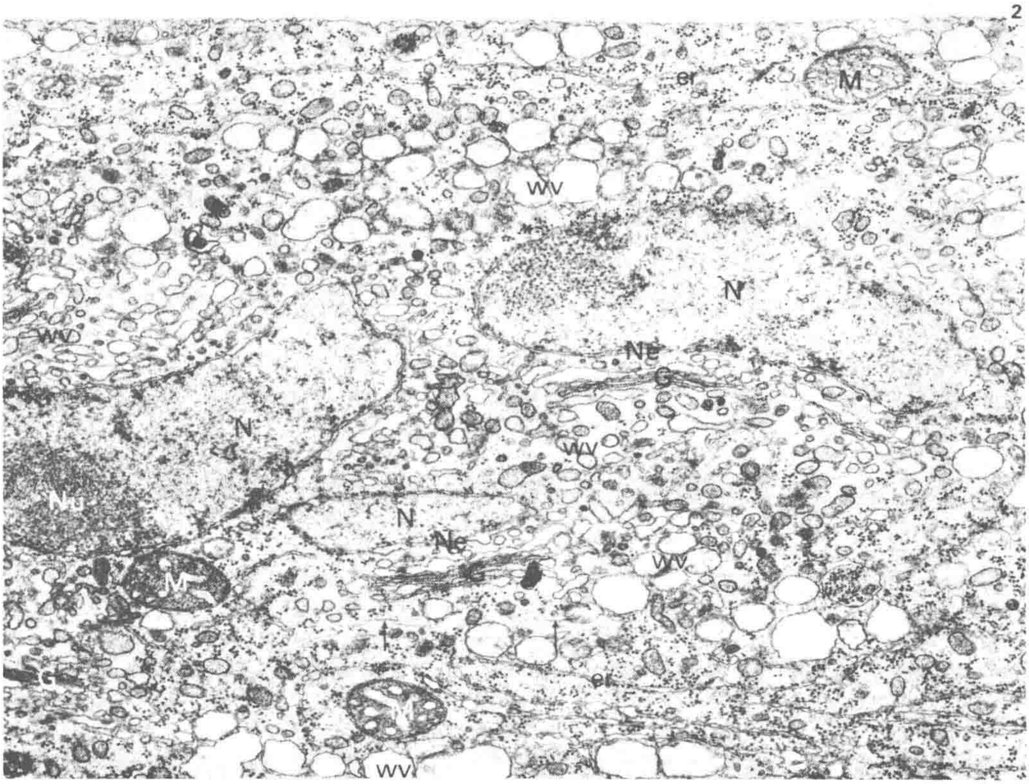

Figure 1.2 *Undivided cell protoplasm*

In a few microorganisms there is no dividing wall or septum separating individual cells. The cell protoplasm is continuous, or undivided. Thus there are multiple nuclei, as shown in this section of a filament of the fungus *Pythium* (the cause of 'damping off', or stem collapse, in plant seedlings). The letters in this figure refer to various organelles, or specialised substructures within the cytoplasm. These are discussed further in Chapter 4. N: nucleus; Nu: nucleolus; G: Golgi body; M: mitochondrion; er: endoplasmic reticulum; wv: vacuole; Ne: nuclear envelope.

Source: A. Beckett, I. B. Heath and D. J. McLaughlin, *An Atlas of Fungal Ultrastructure*, Longman, London, 1974

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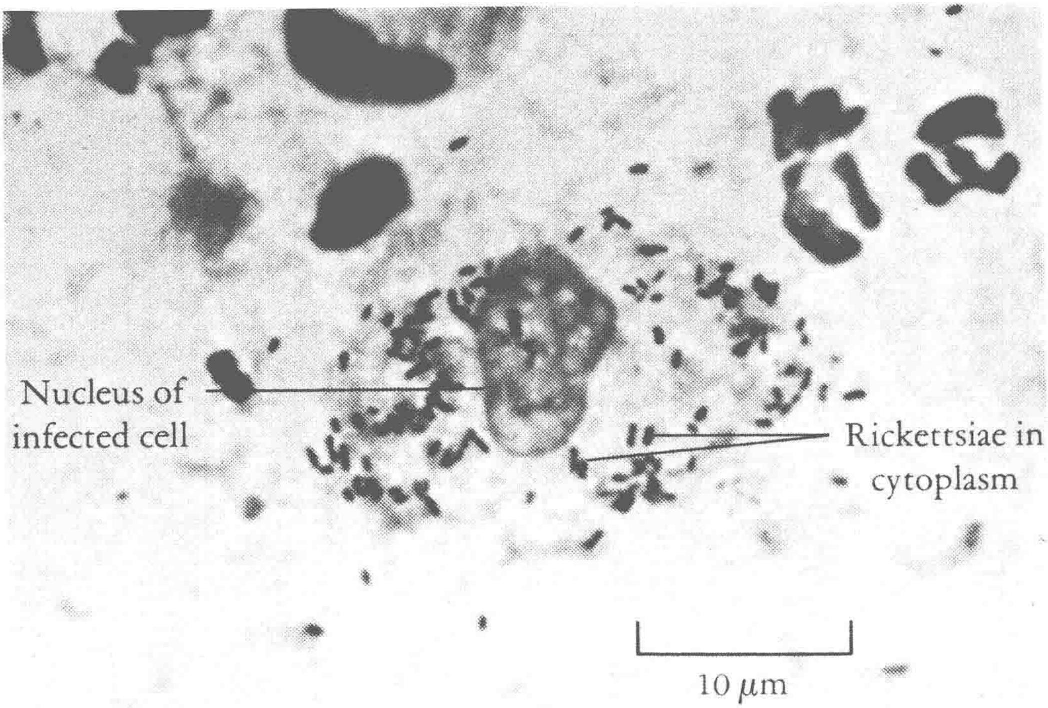


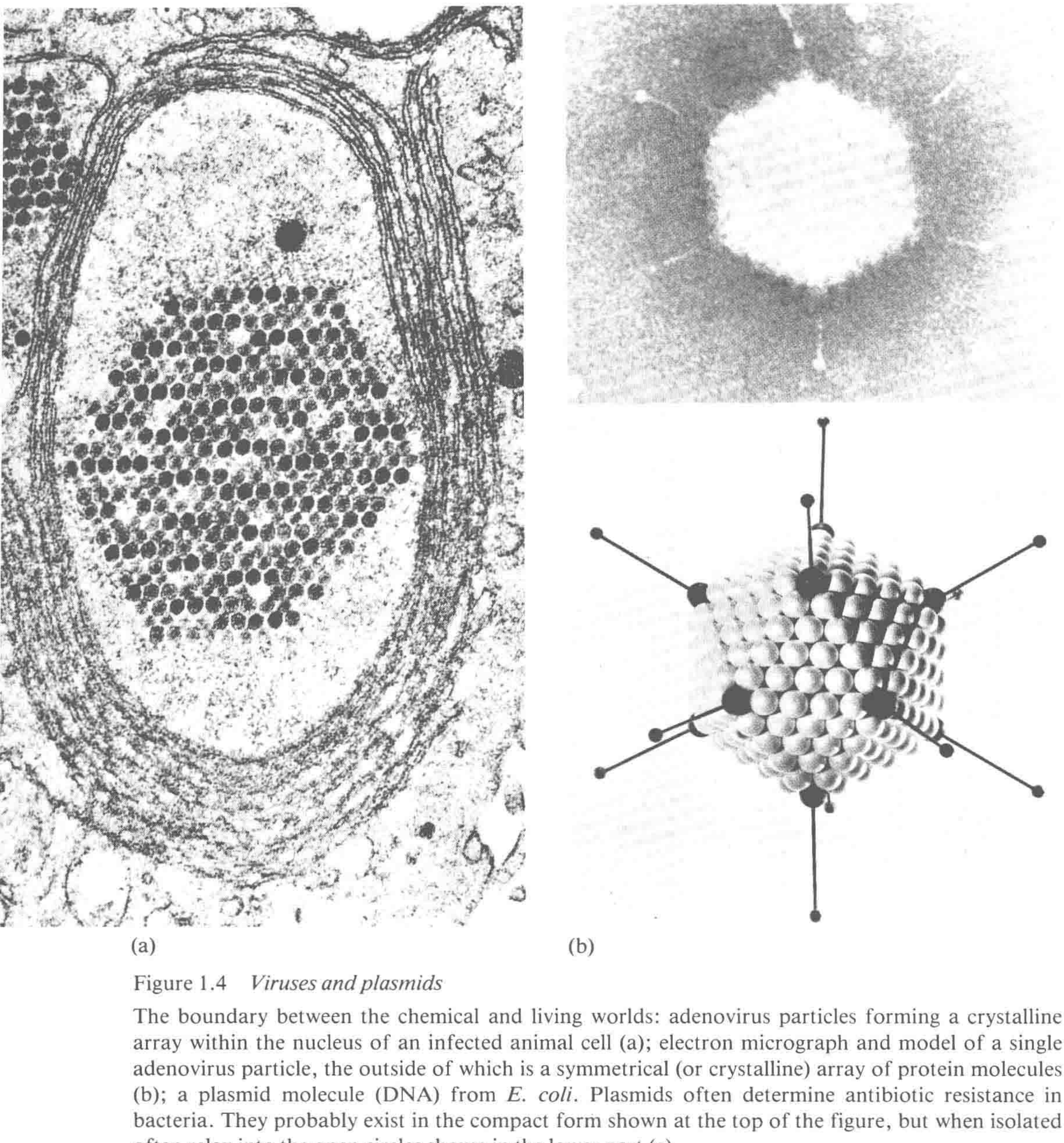
Figure 1.3 *Cells which live within other cells*

The bacterium *Rickettsia rickettsii*, which causes Rocky Mountain spotted fever, is an obligate intracellular parasite, requiring cellular substances which cannot by supplied by 'inanimate' growth media. Therefore it can only be grown within another living cell; it cannot be grown as a pure culture. It nevertheless has many of the metabolic and structural features of a bacterium, which distinguishes it from viruses and plasmids which are also obligate intracellular parasites.

In this illustration, the stained *Rickettsia* cells can be seen within the cytoplasm in chick embryo cells.

Source: C. L. Wisseman et al, *Infection and Immunity*, Vol 14, 1972, p. 1052

- 'undivided' cell material, existing as a continuum of cytoplasm, nuclei and organelles within a large cell-like structure; many examples of this occur among the filamentous fungi and slime moulds (Figure 1.2)
- 'apparent' cells, which have most of the structural features of the normal bacterial cell, but which cannot exist other than within host (usually animal) cells; these are said to be obligate intracellular parasites and can grow only within larger, more complex cells; examples are the chlamydias and rickettsias, both important agents of disease (Figure 1.3)
- viruses (and plasmids) which, like the obligate intracellular parasites, proliferate only within another living cell, but do not have the basic structural features of cells (Figure 1.4). Viruses appear to lie at the dividing line between living and non-living things; plasmids are even simpler. Each will be discussed in more detail in the next section and in Chapter 6.



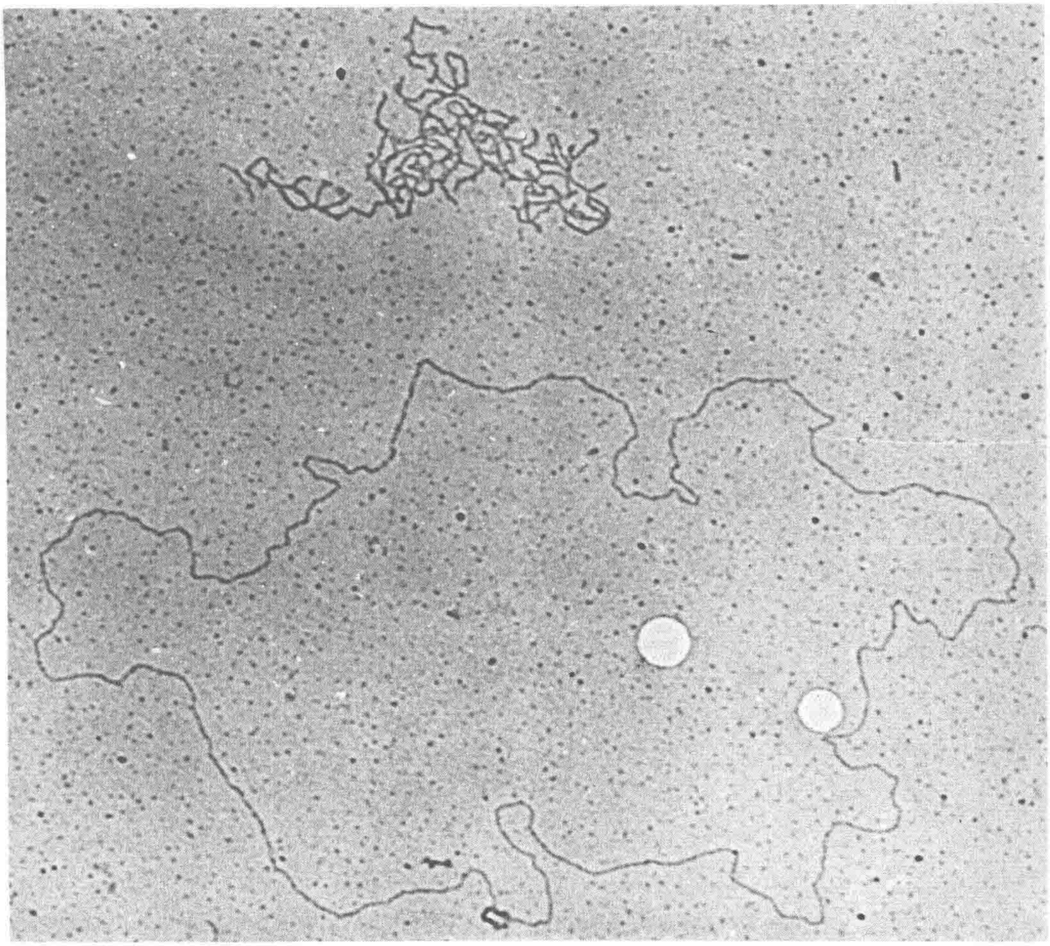
(a) (b)

Figure 1.4 *Viruses and plasmids*

The boundary between the chemical and living worlds: adenovirus particles forming a crystalline array within the nucleus of an infected animal cell (a); electron micrograph and model of a single adenovirus particle, the outside of which is a symmetrical (or crystalline) array of protein molecules (b); a plasmid molecule (DNA) from *E. coli*. Plasmids often determine antibiotic resistance in bacteria. They probably exist in the compact form shown at the top of the figure, but when isolated often relax into the open circles shown in the lower part (c).

Viruses and plasmids are 'organisms' which can proliferate only within living cells; i.e. like *R. rickettsii* in Figure 1.3, they are obligate intracellular parasites. Unlike rickettsias and other obligately parasitic cells, however, viruses consist only of nucleic acid plus protein (plus a membrane envelope sometimes); plasmids consist only of nucleic acid. Virus particles can be crystallised in some cases, and stored like many ordinary chemical substances.

Sources: (a) C. R. Goodheart, *An Introduction to Virology*, W. B. Saunders, Philadelphia, 1969
(b) J. Valentine and R. C. Pereira, *Journal of Molecular Biology*, Vol 13, 1965, p. 12



(c)

Structure of the living cell

Techniques for examining, measuring and describing the features of cells are of two types:

- microscopic, using either the light or the electron microscope. Light microscopes have the advantage that live cells can be examined, though the useful magnification (up to 1000-fold) that can be used is low, relative to the electron microscope. The electron microscope is enormously powerful, and as much as 100 000-fold magnifications are readily attainable. The electron microscope has the disadvantage that cells can only be seen dead by virtue of the fixing, staining and sectioning procedures which are essential preliminaries to its use. A comparison of the relative sizes of representative micro-organisms is given in Figure 1.5
- biochemical analysis to give a measure of the amounts and functions of different chemical substances and complexes which are found in living cells. Biochemical analyses are generally applied after cells have been disrupted or disintegrated, and the constituent, structural components and molecules within have been sorted or separated.

By putting together the information provided by these two general approaches, a detailed picture has been built up of the size, structure and composition of most types of living cells, whether microorganisms or from macroorganisms.

Despite what was said earlier about the similarity of biochemical processes and composition across the living kingdoms, a basic division of cell types can nevertheless be seen amongst existing organisms — prokaryotes and eukaryotes. The differences between these are important for medicine, both in terms of identification of pathogens and in the treatment of the diseases they cause.

The basic structure and composition of prokaryotic and eukaryotic microbes will now be described, and these will be compared with viruses and plasmids.

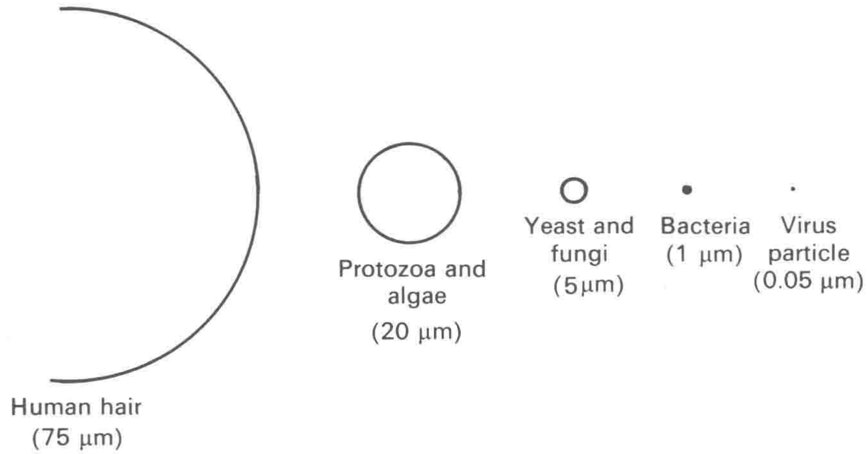


Figure 1.5 *The size of microorganisms*

The circles have been drawn as 700-fold magnifications; the actual dimensions are shown below the circles. This magnification can be achieved using a light microscope with an oil immersion lens. Internal cellular detail can just be discerned in protozoa, algae, yeast and fungi at this magnification, but to resolve internal detail of bacterial cells and virus particles requires an electron microscope. Electron microscopes magnify 100 000 times or more.

The dimensions shown are meant to be representative only; there is considerable variation within groups of organisms. The general trend in size is the significant point. Plasmids are not shown because they consist of a single molecule of circular DNA which is very long when fully extended (100 μm or so), but is smaller than a virus particle when coiled tightly (less than 0.01 μm).

Eukaryotes, prokaryotes, viruses, and plasmids

The prokaryotic cell structure is simple, and is thought to be similar to that of the earliest types of cells which arose on Earth about three billion years ago. Primitive cells of this sort, in one way or another, in turn gave rise to modern prokaryotic bacteria and to eukaryotic cells of fungi, plants and animals, including the microscopic fungi, algae and protozoa.

The prokaryotic cell

The major structural features of the modern prokaryotic cell (exemplified by modern bacteria and blue-green algae) are summarised in Table 1.1 and Figure 1.6. The cell wall (a few exceptions do not have this structure) confers shape and rigidity on the cell and serves to retain the cell membrane and its cytoplasmic contents within a limited space. The concentrations of small and large molecules within all living cells are such that osmotic uptake of water would result in the cell filling with water and bursting if the cell was not constrained in the pressure jacket of the cell wall. The cell membrane is quite permeable to water, and is selectively permeable to a great variety of other small (and sometimes large) molecules, such as salts, sugars, and amino acids which are taken up or extruded by the cell. The cell membrane is sometimes invaginated to form finger-like protrusions or whirls deep within the cytoplasm of the cell. The intracellular membranes or mesosomes thus formed provide surfaces upon which many important cell functions take place, for example energy production and DNA synthesis (Chapter 3). In photosynthetic prokaryotes, such as the blue-green algae, photosynthesis occurs on such intracellular membranes.

The nuclear region is that part of the cell containing the DNA of the chromosome. In bacteria such as *Escherichia coli*, the chromosome is a single circular molecule of double-stranded, helical DNA. The DNA of the chromosome may be attached to a mesosome when new DNA is being synthesised.

Surrounding the nuclear region and filling most of the cytoplasm are small particles called ribosomes on which protein synthesis occurs. Other special structures are sometimes seen within prokaryotic cells; these may be storage granules for phosphate, sulphur, lipid or other substances. In some bacterial cells, spores may form within the wall of the cells, but this occurs in only a few instances.

The eukaryotic cell

By contrast, the eukaryotic cell is a great deal more complex in structure (Figure1.1(a)–(c), Tabel 1.1, Figure 1.7). The increase in complexity is due primarily to the presence in the cytoplasm of organelles, specialised substructures which carry out particular cellular functions, or act as storage systems for lipid, protein or carbohydrate. Cell walls are present among only certain types of eukaryotic cells (those of plants and fungi) and are not found around animal cells. Osmotically induced bursting is countered by wall-less cells, either by the cells living only in an environment of precisely controlled composition which does not induce water uptake (e.g. the cells of organs and tissues in man and animals, bathed in lymph or blood), or by possession of 'pumps' which transfer water out of the cell as rapidly as it is taken up (e.g. in free-living eukaryotes such as amoebae).

A cell membrane is present, and this may be extensively invaginated to form the endoplasmic reticulum, a branched membrane system permeating extensively throughout the cytoplasm. Ribosomes may be bound to the surface of the endoplasmic reticulum, or they may occur singly or in groups free in the cytoplasm. The nucleus is surrounded by a double membrane with distinct openings or pores. The DNA in eukaryotes is found mostly in the nucleus, complexed with special proteins to form chromatin, and this may be seen as darker patches inside the nucleus. Chromatin condenses during cell division to form individual linear chromosomes, but these are not seen in non-dividing cells. The nucleolus is a special region of chromatin within the nucleus that is much darker than other chromatinic areas.

Table 1.1 Features of eukaryotes, prokaryotes, viruses and plasmids

	Eukaryote	*Prokaryote*	*Virus*	*Plasmid*
Cell or particle size	generally 10 micrometres (μm) or more	generally 0.5–5.0 μm	generally 0.01–0.1 μm	uncertain because folding of DNA molecule may vary — probably 0.01–0.1 μm
Cell wall	present in plants, fungi, yeasts, not in animals	generally present	none	none
Cell wall material	cellulose (plants, some fungi); chitin, polymeric sugars in many fungi and yeasts	peptidoglycan	none	none
Cell membrane	present	present	present only in certain cases	none
Structure within cell	many organelles form many different compartments, e.g. nucleus, mitochondria, chloroplasts, endoplasmic reticulum, golgi apparatus, lysosomes, vacuoles	subcellular structures and compartments rare; exceptions are mesosomes, thylakoids, gas vacuoles, storage granules	none	none

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