What is Microbiology?

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I. Introduction

Unlike the physical sciences, biology can be subdivided in two planes: one organismal, the other functional. Organismal subdivision produces disciplines that deal with specific evolutionary branches of the biological world; for example, ornithology, entomology, mycology, bacteriology. Functional subdivision produces disciplines that cross organismal boundaries, such as cytology, genetics, and biochemistry.

Microbiology is seemingly an organismal discipline, since it is

concerned with the properties of small forms of life, or microorganisms. However, the term 'microorganism' does not define a well-circumscribed evolutionary group. According to *Webster's New International Dictionary*, a microorganism is 'any organism of microscopic (also in a broad sense, ultramicroscopic) size; applied especially to bacteria and protozoa'. Let us follow this lexigraphic lead, and see where it takes us.

The human eye cannot resolve an object less than 1 mm in diameter, and can perceive very little structural detail in objects an order of magnitude larger. Microscopic examination is therefore essential either for the very perception, or for the determination of gross structure, of any organism that is 1 mm or less in its largest dimension. Such an organism can be reasonably described as a 'microorganism'.

With very rare exceptions, cells do not have diameters in excess of 1 mm. Consequently, unicellular organisms fall into the microbial category. These include nearly all bacteria and protozoa, as noted by *Webster*. Another class of organisms which are unquestionably microorganisms are the viruses. The only stage of viral development that can be structurally defined is the infectious particle or virion, and the size range of virions extends from the lower limits for cells (0.2–0.3 μm in diameter) to objects two orders of magnitude smaller. The resolution of most virions is thus possible only by electron microscopy, which accounts for the parenthetic proviso ('ultramicroscopic') in Webster's definition.

Some members of the fungi (e.g. yeasts) and of the algae (e.g. diatoms, photosynthetic flagellates) are unicellular and can therefore be construed without ambiguity as microorganisms. Difficulties arise, however, with other members of these two taxonomic groups. Many fungi are coenocytic mycelial organisms, and there is no fixed limit to the size which can be attained during vegetative growth of a single individual. Indeed, some basidiomycetes may produce a mycelium as much as 50 metres in diameter, from which there develop, under conditions favourable for fructification, dozens of fruiting bodies, each of macroscopic dimensions. A similar problem confronts us in the algae. Some algae are either coenocytic or multicellular, and mature individuals may attain a size considerably larger than that of many flowering plants. Strict adherence to the dictionary definition would force us to conclude that some algae and fungi fall in the domain of microbiology, whereas others do not. On the other hand, certain metazoan animals (rotifers and some nematodes) may never exceed 1 mm in their largest dimension, even though they are not commonly considered as organisms that form part of the domain of microbiology.

As this analysis shows, the concept of a 'microorganism' is highly artificial; it is at the same time remarkably broad, in the sense of covering the protozoa, the bacteria, and the viruses, three groups which

differ profoundly in their biological properties; and unduly restrictive, in the sense of cutting through reasonably well-defined natural groups, such as fungi and algae. Microbiology is accordingly not comparable to most disciplines of organismal biology. Nevertheless it is without question a branch of biology that possesses both unity and coherence. These are derived from the fact that it possesses some of the attributes of a functional discipline: the diverse taxonomic groups that fall into the microbiological domain are all susceptible to analysis by a special methodology. It is the methods of microbiology that determine, in the last analysis, the kinds of organisms that constitute its biological subject matter: viruses, bacteria, protozoa, fungi, and algae.

II. The Methods of Microbiology

Microorganisms are ubiquitous in the biosphere; every natural habitat contains an extremely diverse microbial population. On very rare occasions, a population that consists predominantly of one type of microorganism may develop, but such microbial 'blooms' are typically localized in both space and time. Although the relative abundances of different microorganisms fluctuate continuously, the heterogeneity of natural populations is normally so great that any given kind of microorganism represents, at most, a very small fraction of the total. This makes it virtually impossible to study the properties of a specific microorganism in its natural habitat. The problem can be solved only by isolating it from the natural habitat, freeing it from all accompanying organisms, and propagating it as a pure strain in a suitable artificial medium which has been sterilized prior to inoculation, and protected from subsequent contamination by the other microorganisms that are omnipresent in the environment.

A. Sterilization

Fundamental to pure culture technique is the preliminary sterilization (and protection from subsequent contamination) of the media, culture vessels, and implements employed in isolating and manipulating pure cultures. Sterilization can be achieved by exposure to lethal agents, either physical or chemical; or, in the special case of solutions, by filtration. Filtration, the only means of sterilizing solutions of highly labile compounds, has an intrinsic limitation. Although filters that will retain all cellular organisms are readily available, filters sufficiently fine to retain even the smallest viruses are not, since some virions have the dimensions of large protein molecules. Filtration can be used to make a solution cell-free, without necessarily making it virus-free.

The most convenient and widely used agent of sterilization is a physical one: heat. The principles of heat sterilization are not always

clearly understood and merit brief discussion. When a pure culture of a microorganism is exposed to heat (or to any other lethal agent) the kinetics of death are typically exponential: a plot of the logarithm of the number of survivors as a function of time gives a straight line, the negative slope of which expresses the rate of killing. These kinetics reflect the fact that, in a homogeneous population, probability alone determines the time of death of any given individual. The death rate defines what *fraction* of the initial population will survive any given period of exposure to the lethal agent; it does not, of itself, provide information as to the *number* of survivors at this time. This is determined by another parameter, the initial population size. The larger the initial population, the larger the number of survivors after any given period of exposure to the lethal agent. Thus, a much longer period of heating is necessary to sterilize 10 litres of a culture medium than to sterilize 10 millilitres, assuming that they both contain microbial populations of the same initial density. Long experience has shown that the endospores produced by certain bacteria are the most highly resistant of all microbial cells; bacterial spore suspensions are therefore frequently used to calibrate heat sterilization procedures.

In the light of the foregoing discussion, the goal of sterilization can be reformulated in a somewhat more sophisticated way: *the probability that the object subjected to treatment contains even one viable surviving cell should be infinitesimally small.* The sterilization procedures employed by microbiologists are designed to meet this goal, and provide a wide margin of safety.

B. Microbial Nutrition and the Design of Culture Media

In order to grow a cellular microorganism, a culture medium must be prepared which contains an adequate supply of nutrients, i.e. the various chemical substances required for the synthesis of cell materials and for the generation of ATP. The culture of viruses is a different problem which will not be further considered here; their growth is dependent on the provision of suitable conditions for the development of the cellular host.

Cellular microorganisms display an extraordinary nutritional diversity, which reflects their extreme diversity in physiological and biochemical respects. Consequently, it is impossible to prepare a universal culture medium, suitable for the growth of all kinds of microorganisms. The design of a suitable culture medium must be worked out for each particular microbial group, taking into account its special physiological and biochemical properties. For bacteria alone, literally thousands of different culture media have been proposed. Nevertheless, certain general principles govern the design of them all.

In the first place, a culture medium should contain a *balanced* mixture of the different nutrients, each being furnished in a relative amount roughly proportional to biosynthetic requirements; some nutrients are required only in traces, others in much larger amounts. This principle is of critical importance, since depletion of any one nutrient, whatever its nature, will arrest growth, and arrest is sometimes preceded by a short period of unbalanced growth which makes the population physiologically abnormal.

Table 1. Approximate elementary composition of the microbial cell[a]

Element	Dry weight (%)
Carbon	50
Oxygen	20
Nitrogen	14
Hydrogen	8
Phosphorus	3
Sulfur	1
Potassium	1
Sodium	1
Calcium	0.5
Magnesium	0.5
Chlorine	0.5
Iron	0.2
All others	~0.3

[a]Data for a bacterium, *Escherichia coli*, assembled by S. E. Luria, in *The Bacteria* (I. C. Gunsalus and R. Y. Stanier, eds.), Vol. I, Chap. 1 (New York: Academic Press, 1960).

The chemical composition of cells, which varies little, provides a useful insight into general nutritional requirements (Table 1). Water is always the principal molecular component (80–90 per cent by weight) of the living cell, and therefore a major essential nutrient. In addition to hydrogen and oxygen (derivable metabolically from water), the dry matter of cells contains four principal non-metallic elements: carbon, nitrogen, phosphorus, and sulphur. It also contains a variety of metals, of which potassium, sodium, calcium, magnesium, and iron are quantitatively the most important. However, several additional metals, present only in traces in cells, play indispensable roles in cellular metabolism, and are therefore essential nutrients. They include manganese, cobalt, copper, molybdenum, and zinc. Although both sodium and chlorine are normally present at fairly high levels in the dry matter of cells, neither of these elements can be demonstrated to be essential for the growth of most microorganisms, with the exception of those which inhabit marine or hypersaline environments. Indigenous marine

microorganisms have readily demonstrable Na^+ and Cl^- requirements, as well as quantitative requirements for Ca^{2+} and Mg^{2+} considerably higher than those of terrestrial and freshwater forms. With the partial exception of Na^+ and Cl^-, all the above mentioned elements are essential nutrients, and must be provided in any culture medium in a suitable chemical form.

All metals, together with phosphorus (as phosphate) can be provided as nutrients in the form of inorganic salts. The nutritional diversity of microorganisms largely reflects the different molecular forms in which four elements – carbon, nitrogen, sulphur, and oxygen – must be provided.

Most photosynthetic microorganisms (exception: some photosynthetic bacteria) can use the most highly oxidized form of carbon, CO_2, as a carbon source. In these groups, ATP is derived from a physical source, by the conversion of light energy into chemical bond energy. Carbon dioxide can also be used as a carbon source by certain groups of non-photosynthetic bacteria (chemoautotrophs), which can couple the oxidation of reduced inorganic compounds (e.g. NH_3, H_2, H_2S) with ATP synthesis.

All other microorganisms belong to the nutritional category of chemoheterotrophs, which require at least one organic compound as a major nutrient, from which they derive cell carbon. This substance also has a second metabolic role, as a source of ATP; it is in part decomposed by a respiratory or fermentative pathway, the operation of which is coupled with ATP synthesis.

Many chemoheterotrophic bacteria and fungi, as well as a few protozoa, can derive both carbon and energy from the metabolism of a single organic compound. Microbial diversity with respect to the organic substances utilizable for this purpose is extreme. *Every naturally occurring organic compound can be used as a carbon and energy source by at least one type of microorganism.* Consequently the number of different organic compounds utilizable by the totality of microorganisms runs into tens of thousands. Not surprisingly the nutritional spectrum of any given microorganism is narrow, relative to the immense total range. Nevertheless, some bacteria possess remarkably wide nutritional spectra; for example, representatives of the genus *Pseudomonas* can use as single carbon and energy sources at least 100 different organic compounds, including sugars, fatty acids, dicarboxylic acids, hydroxyacids, aminoacids, amines, benzenoid compounds, and sterols. Other bacteria have extremely limited and specialized nutritional spectra; for example, one physiological group, the obligate methylotrophs, can use only two carbon and energy sources: methane and methanol. Methane cannot be utilized by any other microorganisms, and methanol is very rarely utilized by members of other microbial groups.

In the cell, nitrogen and sulphur occur principally in a reduced organic state, as amino ($R{-}NH_2$) and sulphhydryl ($R{-}SH$) compounds, respectively. Many microorganisms can use as sources of these elements the anions NO_3^- and SO_4^{2-}; their incorporation into organic form within the cell is preceded by a reduction to ammonia and sulphide, respectively. Microorganisms unable to perform one of these reductions must be furnished with either ammonia (or sulphide) as a nitrogen (or sulphur) source. Inability to reduce nitrate is relatively common; inability to reduce sulphate is much rarer. Many bacteria (but not other microorganisms) can use N_2 as an inorganic nitrogen source.

Simple nutrient requirements necessarily reflect a high degree of biosynthetic ability. A microorganism able to grow at the expense of a single carbon compound, nitrate, and sulphate, must be able to synthesize from these three nutrients a wide diversity of metabolic intermediates, all essential for cellular function. They include the coenzymes, as well as the monomers required for the synthesis of proteins, nucleic acids, lipids, and polysaccharides. If an organism does not possess the enzymic machinery necessary for the synthesis of any one of these coenzymes or monomers, the compound in question (or its immediate metabolic precursor) becomes an essential nutrient, and must be furnished in the growth medium. Such nutrients, individually required in quantities that are small relative to the principal carbon source, are termed *growth factors.* Growth factors can be divided by virtue of their chemical structures and biological functions into three categories: amino acids, the building blocks of proteins; purines and pyrimidines, the building blocks of nucleic acids; and vitamins, a chemically diverse array of compounds, each of which is a metabolic precursor of a particular type of coenzyme.

In microorganisms that require growth factors, the number and nature of the requirements vary widely. For many microorganisms, the requirement can be met by the provision of a single growth factor, for example, a specific amino acid or vitamin. Other groups of microorganisms have extensive growth factor requirements; this is an evolutionary expression of multiple losses of biosynthetic capacity, resulting from existence in ecological niches where these biosynthetic intermediates are readily available in the external milieu. Among bacteria, the most extreme example of growth factor dependence occur among the lactic acid bacteria. Some species of this group have absolute requirements for as many as 16 of the 20 amino acids that enter into the composition of proteins; four purines and pyrimidines, and numerous vitamins. This nutritional complexity reflects the nutrient-rich natural habitats of lactic acid bacteria, which develop in decaying plant materials, in milk, and in the body cavities of animals. Many protozoa have growth factor requirements of equivalent complexity. This often reflects their predacious mode of life (phag[illegible]phy);

such protozoa normally use as food sources smaller microorganisms, which they ingest by phagocytosis, and digest within intracellular vacuoles.

Even when the precise growth factor requirements of such microorganisms have been determined, the preparation of a chemically defined medium for their cultivation is rarely attempted. There is a much simpler and more expeditious solution: the preparation of a *complex* medium, which contains (in addition to the necessary minerals and a suitable organic carbon and energy source) a product of natural origin rich in growth factors, but of undefined chemical composition. Yeast extract and extracts of plant and of animal tissues, are often used for this purpose. Phagotrophic protozoa can be conveniently grown either as two-membered cultures with an appropriate microbial prey, or as pure cultures, furnished with a heat-killed suspension of the prey. This is often necessary, because many phagotrophic protozoa grow poorly or not at all at the expense of dissolved nutrients, and appear to require food materials in particulate form.

The role of oxygen in microbial nutrition requires special discussion. Although oxygen can be derived metabolically from water, all organisms that obtain energy from oxygen-linked respiration must also be furnished with another molecular form of this element, O_2, essential as a terminal electron acceptor. Organisms of this physiological type (*strict aerobes*) are widespread among bacteria, fungi, and protozoa. However, some fungi (e.g. many yeasts) and bacteria (e.g. members of the enteric group) are facultative aerobes, since they can obtain energy from either the fermentative or the respiratory dissimilation of organic compounds, and thus can grow in the absence of oxygen, provided that they are furnished with a *fermentable* organic substrate. The proviso is important: a facultative anaerobe such as the bacterium *Escherichia coli* behaves as a strict aerobe if provided with a substrate (e.g. acetate or lactate) which can be metabolized only through the respiratory pathway; on the other hand, it behaves as a facultative anaerobe if the organic substrate is a fermentable sugar.

Molecular oxygen is a very reactive compound, and all organisms that live in contact with air possess enzymic devices to prevent the accumulation in the cell of the highly toxic derivatives formed from it. The most damaging derivative is the superoxide free radical, O_2^-. Organisms that can tolerate exposure to molecular oxygen contain an enzyme, superoxide dismutase, which eliminates the free radical by the reaction:

$$2O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2$$

Most organisms also contain catalase, which decomposes the much less toxic product, hydrogen peroxide, to oxygen and water:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

However, many microorganisms (mainly bacteria, and a few protozoa) do not possess these enzymic protective devices, and as a result are very rapidly killed by exposure to molecular oxygen. The cultivation of such *strict anaerobes* requires special precautions to exclude even transient contact of the organisms with air. In the biosphere, strict anaerobes inhabit ecological niches to which oxygen never penetrates, such as the sediments of lakes and oceans and the intestinal tract of animals. Although most strict anaerobes have a fermentative mode of energy-yielding metabolism, some photosynthetic bacteria also belong to this physiological category.

The role of light in microbial growth also calls for additional comment. The mechanisms of photosynthesis and the light-harvesting pigments associated with the process in microorganisms are highly diverse. All algae share with higher plants the ability to perform *oxygenic photosynthesis*, which can be represented by the overall equation:

$$CO_2 + 2H_2O \xrightarrow{\text{light}} (CH_2O) + H_2O + O_2$$

The photochemical processes associated with this mode of photosynthesis lead, in addition to the formation of ATP, to a photochemical splitting of water:

$$2H_2O \xrightarrow{\text{light}} 4[H] + O_2$$

The photochemically derived reductant, NADPH, symbolized above as [H], serves in conjunction with ATP to mediate conversion of CO_2 to organic cell material, symbolized above as (CH_2O). The same mode of photosynthesis exists in one large group of bacteria, the cyanobacteria. All these photosynthetic microorganisms have light-harvesting pigments which absorb in the visible spectral range (roughly, 400–700 nm).

Two groups of bacteria, the purple and green bacteria, perform a less complex version of photosynthesis, termed *anoxygenic photosynthesis* because it is never accompanied by a photochemical cleavage of water, and can therefore not give rise to the formation either of oxygen or of a photochemically generated reductant. Anoxygenic photosynthesis generates only ATP, and the reducing power necessary for CO_2 assimilation must be generated enzymically, from a chemical source other than water. Many purple and green bacteria can use H_2S for this purpose. The over-all reaction for the first step of sulphide oxidation can be represented by the photosynthetic equation:

$$CO_2 + 2H_2S \xrightarrow{\text{light}} (CH_2O) + H_2O + 2S$$

Many purple bacteria and a few green bacteria can also use photochemically derived ATP to perform a direct photoassimilation of organic substrates, a reaction which can be approximately represented

as:

$$\text{Organic substrate} \pm CO_2 \xrightarrow{\text{light}} (CH_2O)$$

These anoxygenic bacterial photosyntheses are anaerobic metabolic processes; many of the bacteria that perform them are, in fact, strict anaerobes. Any organism that performs oxygenic photosynthesis is of necessity an aerobe, in the sense that it must be able to tolerate the presence of molecular oxygen.

Another distinctive property of the purple and green bacteria is the possession of light-harvesting pigment systems which absorb very largely outside the visible range, in the near infrared region (750–1000 nm). This appears to be an evolutionary adaptation designed to avoid direct competition with oxygenic phototrophs for solar radiant energy. Because of their anaerobic propensities, purple and green bacteria are mostly confined to the oxygen-free, subsurface layer in natural bodies of water, and therefore have to perform photosynthesis with wavelengths of light that are transmitted through the cells of oxygenic phototrophs, present in the overlying oxygen-rich water layer. When an artificial light source is used for the growth of purple and green bacteria, it should be incandescent (i.e. with high emission in the near infrared). Fluorescent lamps, excellent energy sources for the cultivation of other photosynthetic organisms, are almost completely ineffective.

C. The Principle of the Enrichment Culture

One very valuable element of microbiological technique which falls outside the domain of pure culture methods, but is often brought into play as a preliminary to their application, is the enrichment culture. It is a device for imposing artificial selection on a heterogeneous natural microbial population, so as to favour the growth of a particular type of microorganism, possessing an ensemble of nutritional and physiological properties determined by the type of selection applied. If the selection is sufficiently rigorous, the microorganism in question, however low its initial abundance, rapidly becomes the predominant member of the population. Its subsequent isolation in pure culture is thereby greatly facilitated.

The power of this method rests on the extraordinary diversity of microorganisms in metabolic, nutritional, and physiological respects. Its development was very largely the work of Winogradsky and Beijerinck, who first systematically applied a wide range of selective conditions to mixed microbial populations, and determined the nature of the organisms that came to predominance in response to each type of selection. Once the biological outcome of a particular set of selective conditions has been determined, the same procedure can be applied

with other natural source materials and (provided that organisms capable of developing under the prescribed conditions are present) the same specific enrichment will result. By making a preliminary series of dilutions of the natural material, it is possible to obtain an estimate of the numerical abundance of any type of microorganism for which there is a specific enrichment method. The enrichment culture technique is therefore also a powerful tool for the study of microbial ecology.

One example will serve to illustrate both the precision and the flexibility of the method. The ability to synthesize the enzyme nitrogenase, and hence to use N_2 as a nitrogen source, is confined to bacteria, although far from universal among them. Hence, if one inoculates the mixed microbial population present in a sample of soil or water into a liquid medium that contains all essential nutrients *except a combined nitrogen source*, and incubates this medium in contact with a gas phase containing N_2, all non-bacterial components of the population, as well as all bacteria unable to synthesize nitrogenase, will be counter-selected. Such an enrichment culture is, in principle, rigorously selective for nitrogen-fixing bacteria. In practice, there is often some carry-over of fixed nitrogen with the inoculum, but one or two further transfers in the same medium eliminate this source of non-selectivity.

Experience has shown that the ability to fix nitrogen occurs in a wide diversity of bacterial groups, which differ markedly in other biochemical and physiological respects. Accordingly, by modifying secondary parameters of the enrichment medium, it is possible to select a particular group of nitrogen-fixers to the exclusion of all others. Table 2 shows some of the specific nutritional variations which can be employed, and the biological outcome of each type of enrichment.

D. The Uses and Limits of Pure Culture Methodology

The cardinal importance of pure culture methods was recognized very early in the development of microbiology, soon after the basic techniques had been worked out by the schools of Louis Pasteur and Robert Koch. A distinguished nineteenth century mycologist, Brefeld, made the point with an aphorism: if one doesn't work with pure cultures, only nonsense and *Penicillium glaucum* (a common contaminating mould) can come out of it. Few (if any) bacteriologists or virologists would disagree with the Brefeldian dictum. However, many phycologists and protozoologists, as well as a few mycologists, still work without recourse to pure culture methodology, and would contend – no doubt justifiably – that useful information about the organisms that fall into their respective domains can be obtained by studying natural populations and even, for fungi and algae, herbarium specimens. It should be noted, however, that the methodological distinction in such

Table 2. Enrichment media and conditions of incubation selective for several different groups of nitrogen-fixing bacteria

Invariant parameters: mineral base, containing Na–K phosphate buffer, $MgSO_4$, $CaCl_2$, and the following trace elements: Mn, Co, Fe, Mo. Incubation in contact with gas phase containing N_2. Temperature 25–30 °C. pH 7–8 unless otherwise stated.

A. Inorganic carbon source (CO_2): source of energy light.
  1. Light of wavelengths 400–700 nm: molecular oxygen present.
     Heterocystous cyanobacteria
  2. Light of wavelengths >700 nm: molecular oxygen excluded.
     a. H_2S present.
        Purple and green sulphur bacteria
     b. Non-fermentable organic compound (e.g. ethanol or acetate) present in addition to CO_2.
        Purple non-sulphur bacteria

B. Organic carbon and energy source: incubation in dark.
  1. Carbon and energy source non-fermentable (e.g. ethanol or acetate): molecular oxygen present.
     a. pH 7 or greater.
        Azotobacter
     b. pH 5 or less.
        Beijerinckia
  2. Carbon and energy source a fermentable sugar (e.g. glucose): molecular oxygen excluded.
     a. No organic growth factors present.
        Klebsiella
     b. B vitamins present.
        Bacillus polymyxa; *Clostridium* spp.

cases clearly provides a line of demarcation between what is microbiology and what is some other kind of biology. A phycologist who confines his studies on algae to field populations and herbarium specimens is not apt to describe himself as a microbiologist; one who studies the same organisms in culture probably will.

One major restriction governs the use of pure culture methodology; *it is applicable only to organisms that can reproduce by asexual means.* Once in pure culture, such an organism can be propagated indefinitely, and on any desired scale, as a pure clone consisting of individuals of genetic near-identity (spontaneous mutations will, of course, introduce minor genetic heterogeneity into the population). *Thus the microbiologist is able to use the clonal population, rather than the individual organism, as an object of study.* The enormous experimental advantages are obvious. He can easily determine properties that are either not determinable at all, or determinable only with the greatest difficulty, on an individual organism of very small size.

Since microbiologists study populations, the principles of population

biology affect almost every aspect of their work. A microbiologist cannot afford to ignore (as many macrobiologists can) the parameters of population growth; the environmental factors that influence growth rate and growth yield; or the play of mutation and selection, which inevitably becomes significant in any large population.

Although the sexual nature of their reproduction precludes the cloning of most animals, the isolation and propagation in pure culture of animal cells and tissues is now a common practice; it represents an extension into the macrobiological domain of techniques originally developed by microbiologists. However, a zoologist who undertakes cell or tissue culture at that point abandons the study of the organism, since such cultures cannot (at least so far) regenerate the animal from which they were derived; they have become permanently separated from the germ line. Cell and tissue culture of higher plants does not always suffer from the same limitation. Experience has shown that clones of plant cells often remain totipotent and can be induced by appropriate treatments to regenerate a mature plant, capable of sexual reproduction. Here, accordingly, pure culture methods can be intercalated with the study of the whole organism.

III. Microorganisms and Major Biological Categories

The notion that all living organisms can be placed in one of two kingdoms, plants and animals, was inherited by nineteenth century biology from the era of Natural History. The gradual realization that it does not accurately reflect biological realities was an outgrowth of the detailed exploration of the microbial world, which got underway in the latter half of the nineteenth century. Study of microorganisms eventually led to the recognition of three primary categories of organisms: viruses, prokaryotes (bacteria), and eukaryotes. The two former categories consist exclusively of microorganisms. The two traditional kingdoms of Natural History, plants and animals, are subgroups of eukaryotes, though it is not easy to fix their limits with precision. The various metazoan phyla can be readily defined as 'animals', and photosynthetic eukaryotes, starting at the level of complexity represented by liverworts, as 'plants'. However, many eukaryotes, including all those of interest to the microbiologist – protozoa, algae, and fungi – cannot be construed either as plants or as animals, without introducing major qualifications into the definitions of these two assemblages, and making arbitrary assignments which lead to the splitting of some evidently natural groups. The relatively simple eukaryotes are best treated as a third major eukaryotic subgroup: the protists. This treatment is also convenient because algae, protozoa and fungi, despite their diversity, really constitute a biological continuum. In the last analysis, a rigorous dividing line cannot be drawn between protozoa and algae, or between protozoa and fungi.

A. The Properties of Viruses

Viruses exist in two different states, one extracellular, the other intracellular. The extracellular state is the infectious particle or virion, inert except when it makes contact with a cell susceptible to infection. A virion consists of one molecule (rarely more) of either DNA or RNA, enclosed by a protein coat, or capsid. The entire structure, a nucleocapsid, is of fixed form and size; and its form is definable in crystallographic terms (a polyhedron or a helix). The polyhedral nucleocapsid of some bacterial viruses bears a helically constructed tail, which plays a specific role in attachment to the host cell and in initiation of infection. The virions of some viruses are enclosed by membranous envelopes, derived from the membrane of the host cell.

The intracellular state of the virus consists of viral nucleic acid, replicated by the metabolic machinery of the host cell. The viral nucleic acid provides, either directly or indirectly, messages for the synthesis of a limited number of viral proteins. These include the subunits (capsomers) from which the capsid is assembled, the tail proteins (if formed), and some enzymes, with specific functions either in the synthesis or the release of virions. Intracellular viral development culminates in the assembly from their molecular constituents of a new population of virions, followed by their release into the external milieu. Viral development is usually but not invariably accompanied by death of the host cell.

Probably all major groups of cellular organisms, both prokaryotes and eukaryotes, can serve as hosts to viruses. Since the host ranges of viruses are relatively restricted, they can be classified in terms of the biological nature of the host, a classification which has led to the distinction of 'animal', 'plant', 'bacterial', and 'fungal' viruses. Experience has shown, however, that a more satisfactory classification can be based on the molecular properties of virions (Table 3).

B. The Common Attributes of Cellular Organisms

In all organisms except viruses, the cell serves as the ultimate unit of structure, function, and growth. The growth of a cell occurs through an orderly increase of all its molecular constituents, leading to progressive enlargement, eventually followed by division into two (or sometimes more) cells.

The genome of a cell is composed exclusively of DNA, the various forms of RNA present in the cell being produced by transcription of specific segments of the genome. Cellular RNAs are divisible into three functional classes, each of which plays a specific role in gene translation: ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and messenger RNAs (mRNAs). Part of the cellular genome consists of so-

Table 3. System for the classification of viruses

Nucleic acid	Capsid symmetry	Naked or enveloped	Size of capsid (nm)[a]	Number of capsomers	Special features	Examples		
						Bacterial	Animal	Plant
RNA	Helical	Naked	17.5 × 300					Tobacco mosaic virus
		Enveloped	9				Myxoviruses	
			18				Paramyxoviruses	
	Polyhedral	Naked	20–25			Coliphage f2		
			28	32			Picornaviruses	Bushy stunt virus
			70	92	Double-stranded RNA		Reoviruses	
DNA	Helical	Naked	5 × 800		Single-stranded DNA	Coliphage fd		
		Enveloped	9–10				Poxviruses	
	Polyhedral	Naked	22	12	Single-stranded DNA	Coliphage φX174		
			45–55	72			Papovaviruses	
			60–90	252			Adenoviruses	
		Enveloped		162			Herpesviruses	
	Binal (polyhedral 'heads', helical 'tails')	Naked	Head: 95 × 65 Tail: 17.5 × 1150			Coliphages T2, T4, T6		

[a] Diameter, in case of polyhedral virion.

Stanier, Adelberg, and Ingraham, *The Microbial World*, 4th ed., © 1976. Reprinted by permission of Prentice-Hall, Inc., Englewood Cliffs, N.J.

called structural genes, which are transcribed as specific mRNAs, and subsequently translated into the polypeptide chains of the numerous (minimum: approx. 500) proteins which enter into the composition of the cell. Most of these proteins are enzymes, endowed with specific catalytic functions, and mediate the complex network of reactions that underlie energy-yielding metabolism and biosynthesis. Metabolic integration, necessary to assure the highly ordered process of growth, is effected by low molecular weight metabolites which act at two levels: to regulate selectively the transcription of structural genes, and to regulate enzyme function.

The cell is always separated from the external milieu by a membrane. This structure, about 8 nm wide, consists of a molecular bilayer of phospholipids, into which are inserted many different proteins. The cell membrane has one universal function; regulation of the passage of solutes between the interior of the cell and the external milieu.

C. The Properties of Prokaryotes

The information necessary to assure the growth and reproduction of a prokaryotic cell is carried by a single circular molecule of double-stranded DNA, the prokaryotic chromosome (see Chapter 11); no exceptions to this rule have so far been discovered. A distinctive property of the prokaryotic genome is the infrequency of repetitive nucleotide sequences; nearly all sequences in the chromosome are unique. The only known exceptions are the genes that encode rRNA; there are several copies of each, scattered randomly over the chromosome. The DNA content of the prokaryotic chromosome ranges from about 5×10^8 daltons (some mycoplasmas) to 5×10^9 daltons (some cyanobacteria). Prokaryotes also often harbour and replicate smaller genetic elements (plasmids), similar in molecular structure to the chromosome. Plasmids rarely contain more than 10 per cent of the amount of DNA in the chromosome, and specify ancillary phenotypic traits, as shown by the fact that their elimination from the cell usually does not impair viability.

The prokaryotic genome is not segregated from the cytoplasmic region by an enclosing membrane. The replicating chromosome and plasmids are attached to the cell membrane; this attachment also determines (in a fashion not yet precisely defined) the separation of daughter genomes. Chromosomal replication can occur continuously throughout the cell cycle, and when growth is rapid, additional rounds of replication can be initiated before completion of the preceding one. Prior to division, a prokaryotic cell thus often contains multiple copies of the genome, either completed or in the course of completion.

Genetic exchange in prokaryotes occurs through unidirectional transfer of DNA from a donor to a recipient cell, effected by conjugation, by transmission in a viral vector (transduction) or by

passage of free DNA through the external milieu (transformation). Only rarely (and only by conjugation) does the recipient cell acquire a complete set of chromosomal genetic determinants from the donor cell. The entry of chromosomal DNA converts the recipient into a transient (and nearly always partial) diploid; the haploid state (normal in all prokaryotes) is reestablished after recombination, and results in the elimination of unrecombined chromosomal alleles. After a transfer of plasmid DNA, the recipient cell often maintains and reproduces the plasmid indefinitely as an autonomous genetic element. However, if nucleotide sequence homologies exist, recombination between the plasmid DNA and the DNA either of the chromosome or of other plasmids already harboured by the recipient cell may occur.

Since recombination is not an obligatory sequel to plasmid transfer, plasmids can be transmitted among, and maintained in, prokaryotes that differ widely in chromosomal genetic constitution (i.e. that belong to widely diverse taxonomic groups). In this respect, the gene pools of prokaryotes are far more open than those of eukaryotes. The horizontal transmission of genetic material has probably been an important factor in prokaryotic evolution, although its role in this respect cannot yet be clearly assessed.

With one probable exception (discussed below), the cell membrane is the only unit membrane system of the prokaryotic cell. In addition to its universal biological role in regulating the transport of solutes, the bacterial cell membrane often has important functions in energy-yielding metabolism. It contains the electron transport system in all aerobic prokaryotes, since oxidative phosphorylation (respiration-linked ATP synthesis) can operate only if the components of this system are integrated into a unit membrane. In two groups of photosynthetic prokaryotes, purple and green bacteria, the photosynthetic electron transport system and the photochemical reaction centres are integrated into the cell membrane. The light-harvesting pigments of purple bacteria are likewise incorporated into the cell membrane, which is accordingly the site of all elements of photosynthetic function in this group of photosynthetic prokaryotes. The light-harvesting pigments of green bacteria are contained in special organelles (chlorobium vesicles), each bounded by a non-unit membrane, and attached to the inner surface of the cell membrane.

Cyanobacteria ('blue-green algae') represent one probable exception to the rule that the cell membrane is the sole unit membrane system in a prokaryotic cell. Their cells contain an extensive intracellular system of flattened membranous sacs, or thylakoids, analogous to the thylakoids of the chloroplast in photosynthetic eukaryotes. The thylakoids are the site of the cyanobacterial photosynthetic apparatus, and appear to be topologically distinct from the cell membrane.

The prokaryotic cell membrane is a barrier to the passage of objects of supramolecular dimensions. As a result, many cellular properties

characteristic of eukaryotes are not possessed by any prokaryotes. These include: the ability to ingest particles or droplets by endocytosis, and to excrete cell products by the complementary process of exocytosis, and the ability to acquire cellular endosymbionts. Since prokaryotes cannot maintain water balance in a hypotonic medium by accumulation and periodic excretion of water in a contractile vacuole (a specialized form of exocytosis), protection of the prokaryotic cell from osmotic lysis in a hypotonic medium can be assured only by passive means, namely by the synthesis of a cell wall that has a tensile strength sufficient to counterbalance turgor pressure.

D. Subdivisions of Prokaryotes

Prokaryotes can be divided into three groups, distinguished by the nature of the cell envelope. The smallest group, the mycoplasmas, do not possess cell walls; the membrane is the outer boundary of the cell. The mycoplasmas are, accordingly, osmotically fragile, and can survive only in an external milieu isosmotic with the cell contents. All other prokaryotes synthesize cell walls, and two primary assemblages can be distinguished by the specific chemical and structural properties of the wall (Table 4). They are termed Gram-positive and Gram-negative bacteria, since the differences between them with respect to wall structure are as a rule correlated with the coloration ('Gram-positive') or non-coloration ('Gram-negative') of intact cells by the Gram stain. This empirical staining method was recognized to be a valuable bacteriological diagnostic tool long before its relation to wall structure was discovered.

One class of wall polymers, peptidoglycans, synthesized only by prokaryotes, is common to both assemblages. The peptidoglycans are heteropolymers, composed of two amino sugars and a small number of amino acids. The monomeric components are cross-linked; consequently, peptidoglycans have the structure of a two- or three-dimensional molecular mesh. They thus confer tensile strength on the wall, and also determine the shape of the enclosed cell. In Gram-positive bacteria, peptidoglycan extends throughout the wall, intermingled with the other types of wall polymers. In Gram-negative bacteria, peptidoglycan is confined to the inner layer of the wall, and the other wall polymers are incorporated into a physically distinct outer wall layer with the fine structure of a unit membrane. This so-called 'outer membrane layer' of the Gram-negative cell wall mimics the cell membrane structurally, but differs profoundly from it in molecular composition.

Table 4. Structural and compositional differences between the cell walls of Gram-positive and Gram-negative bacteria

	Gram-positive bacteria	Gram-negative bacteria
Fine structure:	Homogeneous, 10–50 nm wide	Inner dense layer, 2–10 nm wide; outer membrane layer, 7–8 nm wide
Location of peptidoglycan:	Throughout	Inner dense layer
Location of other wall polymers:	Throughout	Outer membrane layer
Nature of other wall polymers:	Teichoic acids; polysaccharides; in some, complex lipids	Lipopolysaccharides; lipoproteins; proteins; phospholipids

Table 5. Distribution of other major properties between the assemblages of Gram-positive and Gram-negative bacteria. None of these properties is universal within either assemblage.

	Gram-positive	Gram-negative
Performance of photosynthesis	–	+
Gliding movement	–	+
Formation of endospores	+	–
Mycelial vegetative growth	+	–

Both the Gram-positive and the Gram-negative assemblages are very large and internally diverse. Nevertheless, a primary separation on the basis of wall structure results in an absolute segregation of bacterial groups distinguished by other major properties, as shown in Table 5. This suggests that the wall provides a marker to distinguish two large evolutionary subgroups among prokaryotes.

The Gram-positive subgroup includes the actinomycetes, which have a mycelial vegetative structure, associated with reproduction by the formation of spores produced from the tips of the mycelial branches; these organisms are prokaryotic structural counterparts of the mycelial fungi. Only certain Gram-positive bacteria produce the specialized resting cells termed endospores. Most endospore-forming Gram-positive bacteria are unicellular, but these resting structures are also produced by a few actinomycetes.

All photosynthetic bacteria – purple bacteria, green bacteria and cyanobacteria – are Gram-negative prokaryotes.

The property of active movement is sporadically distributed among prokaryotes, which can move by two different means, swimming and gliding. Swimming movement, displayed by some members of both the Gram-positive and Gram-negative assemblages, is effected by very thin filiform proteinaceous organelles (bacterial flagella), which extend through the cell wall (except in one Gram-negative group, the spirochaetes, where they lie between the inner and outer cell layers).

During cell movements, the flagella rotate; the motive force is derived from a structure within the cell, the flagellar motor, to which the flagellum is anchored. Gliding movement, which is confined to certain groups of Gram-negative prokaryotes (myxobacteria, flexibacteria, some green bacteria, and cyanobacteria) is not associated with the presence of visible external organelles, and contact of the cell with a solid surface is necessary for its expression. Its mechanism is still not understood.

The great internal diversity of both the Gram-positive and the Gram-negative assemblages suggests that each is composed of a large number of highly isolated evolutionary lines, which diverged early in the course of cellular evolution. This inference has been confirmed in recent years by molecular evidence. In both assemblages, there is an extreme diversity of mean base composition of the DNA; divergence with respect to this molecular parameter is a sure expression of long evolutionary separation. There are also major divergences within each assemblage with respect to rRNA nucleotide sequences, encoded by genes that are very highly conserved during evolution. The use of these and other molecular probes is gradually permitting the recognition of certain large clusters of evolutionarily related bacteria; for example, a major cluster centred around the lactic acid bacteria in the Gram-positive assemblage, and another major cluster centred around the enteric group and the vibrios in the Gram-negative assemblage. Despite these important advances in our understanding of the evolutionary structure of the prokaryotes, it is unlikely that a complete picture of their evolutionary interrelationship will ever be attainable.

E. The Properties of Eukaryotic Protists

Although the purpose of the following discussion is to summarize the cellular properties that distinguish eukaryotic protists from prokaryotes, it must be emphasized that the eukaryotic realm is indivisible at the cellular level. In terms of fundamental cellular properties, what is true collectively for fungi, protozoa, and algae is also true for elephants and flowering plants. In effect, plants and animals represent two major eukaryotic evolutionary lines distinguishable from eukaryotic protists only by their greater organismal complexity, brought about by elaborate, genetically determined developmental programmes, which unfold in the course of their ontogeny.

A basic differential property of the eukaryotic cell is its internal differentiation into two (or sometimes) three regions, each the site of part of the genetic information required to specify cellular properties, and each containing a separate system for the transcription and translation of genetic messages. These are: the nucleo-cytoplasmic region, the mitochondrion, and (in photosynthetic eukaryotes) the chloroplast.

1. Chloroplasts and Mitochondria

The mitochondrion and the chloroplast, both enclosed by the nucleo-cytoplasmic region, are separated from it by organellar membranes. Each is genetically semiautonomous, since it contains a small genome which specifies some (but not all) organellar properties. The chloroplast genome size is approximately 10^8 daltons, the mitochondrial genome, approximately 10^7 daltons; the former accordingly has an information content some 10–20 per cent of that of the smallest known prokaryotic chromosome, the latter, an order of magnitude less. The molecular structure of these organellar genomes and of the systems of organellar transcription and translation associated with them are markedly different from the corresponding functional elements located in the nucleo-cytoplasmic region.

The mitochondrion is the specific site in a eukaryotic cell of the machinery of terminal respiration; the enzymes of the tricarboxylic acid cycle and the respiratory electron transport system. Its function is to provide much of the ATP (derived from oxidative phosphorylation) required for the maintenance of cellular function. It is a well-nigh universal component of eukaryotes. A few protozoa and fungi, which have become adapted to life in anaerobic niches, do not contain mitochondria and are entirely dependent on glycolysis for ATP synthesis.

The chloroplast has a considerably more complex metabolic role, as suggested by its greater genome size. It contains the light-harvesting pigments, the photochemical reaction centres, and the photosynthetic electron transport system necessary for the generation of ATP and reductant (NADPH) through the reactions of oxygenic photosynthesis. These products of photochemical energy conversion are largely used to mediate biosynthetic reactions within the chloroplast itself; notably, the conversion of CO_2 to sugar phosphates through the reductive pentose phosphate cycle; the reduction of oxidized inorganic nitrogen compounds (nitrite, possibly also nitrate) to ammonia, and the incorporation of the latter into glutamine; the reduction of sulphate to H_2S, and its incorporation into organic sulphydryl groups. The relatively simple nutritional requirements of photosynthetic eukaryotes are thus in large measure made possible by the biosynthetic machinery located within the chloroplast.

Chloroplasts and mitochondria contain internal unit membrane systems, topologically distinct from the surrounding unit membrane of the organelle; in both, they are the sites of the machinery of electron transport and ATP synthesis, and (in chloroplasts) of the other elements of the photosynthetic apparatus. The role of the external organellar membrane is thus largely if not entirely to regulate solute transport between the interior of the organelle and the surrounding nucleo-cytoplasmic region.

2. *Nucleo-cytoplasmic Region: Genetic Aspects*

Most of the DNA of the eukaryotic cell is located within the membrane-bounded nucleus; it specifies both properties of the nucleo-cytoplasmic region, and some properties expressed specifically in chloroplasts and mitochondria. The nuclear DNA is dispersed over a number of separate structures, the eukaryotic chromosomes, each of which carries only part of the total genetic message. The haploid chromosome number is fixed, and is a fundamental property of each species: it ranges from 3 to over 100.

In terms of its molecular composition, the eukaryotic chromosome is a much more complex object than either the prokaryotic chromosome or the genome of a chloroplast or mitochondrion. It is an elongated structure, probably containing a continuous longitudinal strand of double helical DNA, firmly associated with a considerable number of specific proteins, both basic (histones) and acidic. This nucleoprotein complex is known as chromatin. A remarkable feature of eukaryotic chromosomal organization is the sequential clustering, on one or more chromosomes, of the numerous identical copies of the genes that encode rRNA. In the interphase (non-dividing) nucleus these particular regions are looped off from the mass of chromatin and associated with specific proteins, to form an intranuclear organelle, the nucleolus, visible by light microscopy. Another distinctive property of eukaryotic chromosomal DNA is the presence of many repeated identical or near-identical nucleotide sequences, some of which do not appear to be transcribed.

Transcription of the nuclear genome is effected by three different polymerases, each specific for the synthesis of one RNA class: rRNA, tRNA, and mRNA. Transcription of structural genes may lead to the formation of polycistronic mRNAs, since adjacent cistrons are not (as in prokaryotes) always separated by chain termination and initiation codons. The polycistronic messages pass into the cytoplasm, where they are translated on the 80S ribosomes into continuous polypeptide chains, subsequently cleaved by specific proteases into functional units (i.e. the shorter polypeptide chains of the different proteins encoded in the polycistronic message).

In eukaryotes, both chromosome replication and nuclear division are closely integrated with the cell cycle, in the sense that they normally occur only once between two succeeding cell divisions. Replication of chromosomal DNA occurs during interphase, being initiated some time after the preceding cell division, and terminated some time before the beginning of the events that lead to the next cell division. The divisional events start with division of the nucleus (mitosis), a process of great morphogenetic complexity, as well as of great diversity in different groups of protists. We shall attempt to summarize here the common denominators.

In the interphase nucleus, the chromosomes are highly extended, and hence not resolvable by light microscopy (exception: dinoflagellates). At the onset of mitosis, they shorten and thicken by coiling, and each chromosome splits longitudinally into a pair of chromatids, joined to one another at only one point, the centromere. During these events, an oriented system of proteinaceous microtubules, assembled from a pool of the monomeric precursor protein (tubulin) present in the cytoplasm, forms in the nuclear region. Some, the polar microtubules, develop from two organizing centres, located on the opposite sides of the nucleus, to form a structure termed the spindle. The organizing centres are located outside the nuclear membrane in biological groups where this structure disintegrates during mitosis; in many protists (e.g. the majority of fungi) where the nuclear membrane persists, the organizing centres are internal to it. A second set of microtubules (chromosomal microtubules) develops from the centromere of each pair of chromatids, extending to the organizing centres, and lying parallel to the polar microtubules of the spindle. The pairs of chromatids become aligned in the equatorial plane of the spindle, the centromeres split, and one copy of each chromosome is pulled towards each organizing centre, through a sliding force exerted between the chromosomal and the polar microtubules. Once chromosome separation is complete, two interphase nuclei re-form, and the microtubular system depolymerizes into tubulin.

Cell division normally begins during the terminal phase of mitosis, in a plane at right angles to the spindle, cutting across the equator. In coenocytic organisms (most fungi, many algae) mitosis is not normally followed by cell division; these organisms consequently consist of a single large, multinucleate protoplast during vegetative development. Only reproductive cells (spores, gametes) are uninucleate; and in some fungi, even spores contain several nuclei.

With exceptions among the fungi, some of which can undergo recombination resulting from the fusion of genetically distinct vegetative nuclei contained in a single coenocyte ('parasexual recombination'), genetic recombination among eukaryotes is always the sequel of a sexual process. It is preceded by the formation of haploid cells of different mating types, known as *gametes*. If they can be distinguished by structural properties (e.g. size, motility), the gametes are termed 'male' and 'female', a terminology derived from the differences in gamete structure characteristic of male and female animals. However, in many protists, the gametes are structurally indistinguishable, those of opposite mating type being termed '+' or '−'. Pairwise gametic fusion is followed by nuclear fusion, to produce a zygote with one diploid nucleus. Genetic recombination occurs subsequently, during the development of the zygote or of a diploid vegetative cell derived from it, and is effected by a special divisional process known as meiosis. Meiosis leads to the formation, from a diploid cell, of four haploid

daughter cells. During meiosis, a random reassortment of the two chromosome sets contributed by the gametes occurs; and genetic recombination is further enhanced by exchange (crossing-over) of homologous regions between each diploid pair of chromosomes. Recombination is thus much more far-reaching than in prokaryotes. Moreover, whereas recombination in prokaryotes leads to the emergence of a unique haploid recombinant genome in the recipient cell, eukaryotic sexual recombination through meiosis always results in the reemergence, in the four haploid products, of all the nuclear genes contributed by both gametes, recombination is reciprocal.

Genetic recombination in eukaryotes is subject to an additional complication: the genetic information carried by mitochondria and chloroplasts is transmitted in these organelles, its inheritance thus being independent of that of the nuclear genome, and subject to different rules, alike of transfer and of recombination.

The relative importance of haplophase and diplophase in the life cycles of eukaryotic protists capable of sexual reproduction varies widely. Predominance of the diplophase, resulting from meiosis immediately preceding gamete formation, brings into operation a genetic phenomenon of profound evolutionary importance, which plays no role in the evolution of prokaryotes: *dominance*. In the diploid state, phenotypic expression of one allele carried by a pair of homologous chromosomes is often partly or wholly repressed. This permits the accumulation of a masked store of genetic variability in diploid eukaryotes.

3. *Nucleocytoplasmic Region: Functional Aspects*

Between the cell membrane and nuclear membrane, the cytoplasmic region is traversed by a complex membranous network termed the endoplasmic reticulum. For the most part topologically irregular, it assumes at one or two sites a more well-defined structure, the Golgi apparatus. This consists of a series of membranous vesicles in parallel array, flattened and elongated at one pole, smaller, more numerous and nearly isodiametric at the other. The organelle, continuously regenerated at the former pole and disassembled at the latter pole, is the site of the synthesis, accumulation and transport of a variety of biosynthetic products: some of the Golgi vesicles contain enzymes, others (in algal and plant cells) fragments of the cell wall fabric. Golgi vesicles that contain materials destined for export from the cell (extracellular enzymes, wall materials) migrate to the cell surface, where their enclosing membranes fuse with the cell membrane, the vesicle contents being discharged to the external milieu. This is the process of exocytosis.

The inv[illegible] process of endocytosis occurs by invagination and

pinching off of a small area of the cell membrane, surrounding either a droplet of liquid (pinocytosis) or a solid particle (phagocytosis) to form a membrane-bounded vacuole which moves into the cytoplasm. Endocytosis underlies two unique and distinctive abilities of eukaryotic cells: the ability to acquire cellular endosymbionts, and the capacity for intracellular digestion. Endosymbionts, once internalized by endocytosis, proceed to grow in the cytoplasmic region, separated from the cytoplasm by the surrounding host membrane. Intracellular digestion of materials ingested as food vacuoles involves the intervention of vesicles derived from the Golgi apparatus (lysosomes and peroxysomes), which coalesce with the food vacuole, and thus introduce into it an array of enzymes, either hydrolytic (lysosomes) or oxidative (peroxysomes). The enzymic breakdown of complex food materials enclosed in the vacuole is followed by diffusion of the soluble products into the cytoplasm. This mode of nutrition, phagotrophy, is possible only in protists that are not enclosed by rigid walls. It is widespread in protozoa, relatively rare in algae, and not displayed by fungi. In fungi, as in prokaryotes, digestion of polymeric nutrients (e.g. proteins, polysaccharides) is extracellular, and is mediated by the secretion of hydrolases.

The nuclear membrane of eukaryotes is distinguished from the other membranes of the nucleocytoplasmic region by its ultrastructure: it is perforated by a regular system of pores. In many though not all protists the nuclear membrane disintegrates early in mitosis, and is reassembled (probably from the endoplasmic reticulum) at the termination of the process.

Accordingly, the different parts of the nucleocytoplasmic membrane system are largely, and perhaps completely, interchangeable. Areas of the cell membrane can be internalized, becoming vacuolar membranes, and vesicle membranes arising from the Golgi apparatus can enter the fabric of the cell membrane as a result of exocytosis. Membrane plasticity and membrane migration are fundamental properties of the nucleocytoplasmic region of a eukaryotic cell.

Implicit in these interchanges of membrane material (but also manifested in many other ways) is the internal mobility of the eukaryotic cytoplasm, often markedly vectorial (e.g. in exocytosis). Internal mobility is usually evident upon microscopic examination of a living eukaryotic cell, being manifested by marked relative displacement of its contents (cytoplasmic streaming). These continuous intracellular movements necessarily imply the existence of an internal motive force, operative even in eukaryotic cells that are immotile, in the sense of being unable to effect translatory movement through or over the external milieu. Most fungi are 'immotile' in this sense, but often display very vigorous cytoplasmic streaming. In protists not enclosed by cell walls (many protozoa and slime molds), direct[illegible] cytoplasmic

streaming permits slow translatory movement of the cell (so-called amoeboid locomotion).

One probable agent of intracellular mobility has been recently recognized as of wide distribution in eukaryotic cells. It is the actinomyosin system: the ensemble of proteins which (in a very highly organized state) are responsible for the contractile properties of vertebrate muscles. Elements of this system have now been identified in the cells of virtually all eukaryotes, including higher plants. Originally presumed to be synthesized only in certain highly differentiated animal tissues, the proteins of the actinomyosin system (or homologues thereof) may well prove to be universal molecular markers of eukaryotic cells.

Another protein which, in polymerized form, participates in some forms of internal eukaryotic cellular movement, and may likewise prove to be a eukaryotic molecular marker, is tubulin. The participation of microtubules composed of tubulin in chromosome movement during mitosis has already been discussed. Tubulin-based microtubular systems have additional functions in the nucleo-cytoplasmic region of eukaryotic cells. They provide the internal structural framework of the eukaryotic cilium or flagellum. This elongated microtubular complex, the *axoneme*, is surrounded by an outpocketing of the cell membrane. It is composed of nine doublet microtubules, surrounding two singlets. Ciliary movement has recently been shown to involve sliding between the two tubules of each doublet, effected by the making and breaking of bridges between them. The axonemal structure is homologous in all protists that synthesize these organelles (many protozoa and algae; aquatic fungi). Both in its machinery and its mechanism, ciliary movement is radically different from prokaryotic flagellar movement.

F. Algae, Protozoa, and Fungi

Among the eukaryotic protists, three major groups are traditionally distinguished: algae, protozoa, and fungi. At first sight, the distinctions seem clear-cut. The distinguishing property of algae is the possession of chloroplasts, which confer on them the ability to perform oxygenic photosynthesis, and to use CO_2 as a principal carbon source. Both protozoa and fungi lack these organelles, and are consequently dependent on organic compounds as sources of both carbon and energy. The traditional distinction between the two latter groups is based on cellular properties: protozoa are unicellular, and the cells are frequently not enclosed by rigid walls: fungi are for the most part coenocytic and are enclosed by rigid cell walls, which typically confer on the enclosed multinucleate coenocyte the structure of a much-branched tubular mycelium. These distinctions are satisfactory, provided t[illegible] one takes into consideration only typical, specialized

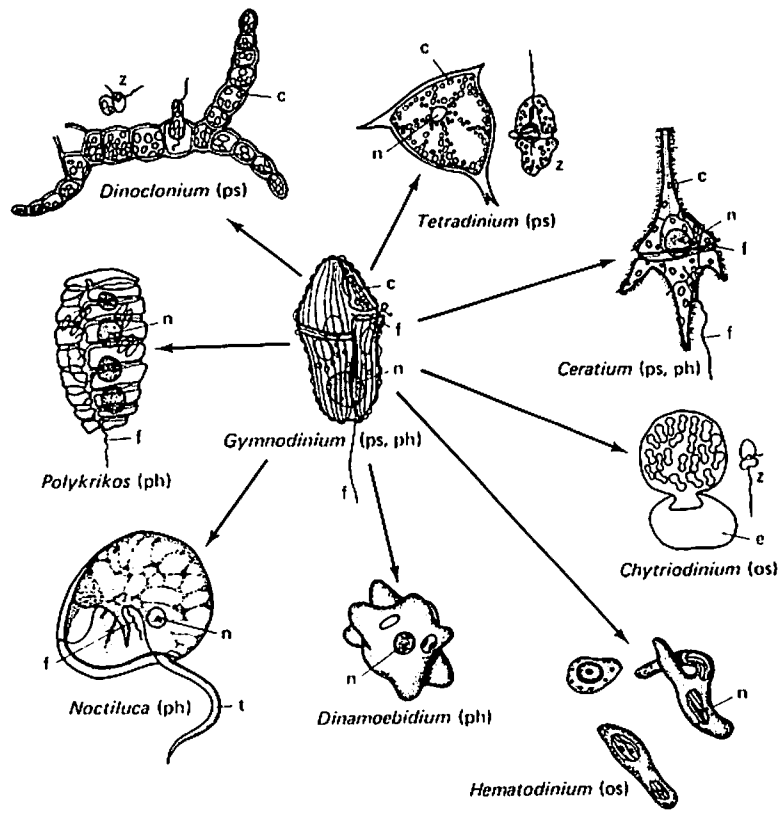

Figure 1. The different evolutionary trends that are represented among dinoflagellates. *Gymnodinium* is a relatively unspecialized photosynthetic dinoflagellate, which is both photosynthetic (ps) and phagotrophic (ph). *Ceratium* is a more specialized photosynthetic dinoflagellate, characterized by a very complex wall with spiny extensions, comprised of many plates. *Tetradinium* and *Dinoclonium* are non-motile, strictly photosynthetic organisms, which reproduce by multiple cleavage to form typical dinoflagellate zoospores. *Polykrikos*, *Noctiluca*, and *Dinamoebidium* are three free-living phagotrophic dinoflagellates. *Polykrikos* is a coenocytic, multinucleate organism, the cell of which bears a series of pairs of flagella. *Noctiluca* has one small flagellum, and bears a large and conspicuous tentacle. *Dinamoebidium* is an ameboid organism. *Chytriodinium* and *Hematodinium* are parasitic dinoflagellates whose nutrition is osmotrophic (os). *Chytriodinium* parasitizes invertebrate eggs and reproduces by cleavage of a large sac-like structure into dinoflagellate zoospores. *Hematodinium* is a blood parasite in crabs: n, nucleus; f, flagellum; c, chloroplast; z, zoospore; e, parasitized invertebrate egg; t, tentacle. (Stainer, Adelberg, and Ingraham, *The Microbial World*, 4th ed., © 1976. Reprinted by permission of Prentice-Hall, Inc., Englewood Cliffs, N.J.)

representatives of each group: a seaweed, a ciliate protozoan, and a basidiomycetous fungus conform perfectly to the differential criteria outlined above. In reality, the situation is more complex, since there are many transitions between algae and protozoa on th[illegible]e hand, and

between protozoa and fungi on the other. Where such transitions exist, the evidence suggests that protistan evolution has always proceeded along similar pathways, as described below.

The stem groups, evolutionarily speaking, of the eukaryotic protists appear to have been a variety of unicellular photosynthetic flagellates, from which two primary lines of divergence have occurred: firstly, maintenance of photosynthetic function (chloroplasts) accompanied by an increase of organismal complexity, leading to evolution of multicellular algae; secondly, loss of photosynthetic function (chloroplasts), leading to unicellular non-photosynthetic organisms of the protozoan type, followed by a secondary increase in organismal complexity, leading to fungi. This evolutionary scenario is revealed with particular clarity in the dinoflagellates, a large group of eukaryotic protists which display certain unique nuclear properties, notably a highly specialized form of mitosis and a failure of the chromosomes to elongate after nuclear division, as a result of which these structures remain resolvable by light microscopy in interphase. Most dinoflagellates also produce motile cells bearing two unequal flagella, one lying in an equatorial girdle, and the other directed posteriorly. These conserved properties provide evolutionary markers which make it possible to detect dinoflagellate affinities in protists that have diverged very markedly in most other respects from the unicellular photosynthetic flagellate pattern. The different evolutionary patterns displayed among dinoflagellates are schematized in Figure 1. They include: (a) typical simple immotile 'algae' (*Tetradinium, Dinoclonium*); (b) highly specialized types of 'protozoa', either free-living predators (*Polykrikos, Noctiluca, Dinamoebidium*) or parasites (*Hematodinium*), and (c) a simple parasitic 'fungus', *Chytriodinium*.

G. Possible Evolutionary Filiations Between Eukaryotes and Prokaryotes

The profound differences between eukaryotic and prokaryotic cells find their expression, at the eukaryotic level, in the nucleo-cytoplasmic region. Chloroplasts and mitochondria, even though their phenotypic properties are in considerable part determined by nuclear genes, are in many respects analogous to prokaryotic cells. In terms of energy-yielding metabolic function, their counterparts are cyanobacteria and aerobic, chemoheterotrophic bacteria, respectively. It is therefore conceivable that each class of organelle arose from free-living ancestral prokaryotes with the same mode of energy-yielding metabolism, which entered into endosymbioses with cellular organisms that already possessed most (if not all) attributes now characteristic of the nucleo-cytoplasmic region of the eukaryotic cell. If so, the initial endosymbioses were followed by far reaching evolutionary modifications *in vivo*. The most important was a reduction in the size of the endosymbiont genome, accompanied by the transfer of many genetic determinants to the genome of the host nucleus. The way in which this feat of genetic engineering might have occurred is mysterious; its adaptive function is evident. It would have assured the permanent subjection of the endosymbionts, still potentially free to escape and resume independent existence as long as they possessed full genetic autonomy. This scenario is a highly plausible one to account for the origin of chloroplasts, which still retain a relatively large genome and a very complex array of metabolic functions. It is perhaps more arguable for mitochondria, which have a far smaller genome and a more restricted array of metabolic functions.

An endosymbiotic origin for chloroplasts and mitochondria provides the most useful evolutionary working hypothesis to account for the properties of these cellular components. This hypothesis implies a prior evolution of the protoeukaryotic cell, embodying the properties of the nucleo-cytoplasmic region. Such a cell would have been dependent on fermentative mechanisms of ATP synthesis, relatively inefficient in terms of energy yield per unit of substrate metabolized. Barring the unlikely possibility of a biphyletic origin of cells, it must be assumed that the protoeukaryote evolved, in the remote past, from a cell of the prokaryotic type, and thus underwent extensive evolution prior to acquiring endosymbionts endowed with respiratory or photosynthetic function. Its divergence from the prokaryotic line could have been initiated by a change of membrane structure, which made possible a primitive type of endocytosis, opening the way to a predacious mode of life. Many of the cytoplasmic properties of the eukaryotic cell – notably the actinomyosin- and tubulin-based systems, and the Golgi apparatus – can be construed as secondary evolutionary adaptations in the context of this mode of life. The invention of tubulin was also a prerequisite for the eukaryotic type of nuclear division; microtubules are associated with nuclear division even in dinoflagellates, where the process is so atypical that it barely qualifies as 'mitotic'.

The unique nuclear properties of dinoflagellates suggest that some of the evolutionary diversity characteristic of contemporary protists may have occurred through divergence at the protoeukaryotic stage. Another possible source of very early divergence could have been the acquisition by already divergent protoeukaryotes of different types of prokaryotic endosymbionts. This possibility is suggested by the differences among chloroplasts (notably with respect to light-harvesting pigments) in the various major algal groups. Only the red algal chloroplast has a pigment system highly homologous with that of contemporary cyanobacteria. The recent discovery of a new group of prokaryotes which perform oxygenic photosynthesis but possess a pigment system of the green algal–higher plant type has provided the

first solid support for the notion that contemporary chloroplast diversity could reflect an ancient divergence at the level of the prokaryotic ancestors.

IV. The Relations Between Microbiology and General Biology

Microbiology was to a very considerable extent the creation of Louis Pasteur. He was primarily responsible for developing its distinctive methodology, as well as for revealing the roles of microorganisms as agents of chemical transformations and infectious disease. During the same period, biology came of age; Darwin imposed order and meaning on the hitherto anecdotal materials of Natural History by reinterpreting them in terms of the theory of evolution based on natural selection. It might therefore have been anticipated that microbiology would take its place, together with other specialized biological disciplines, within the conceptual framework of post-Darwinian biology. This did not occur: for almost a century, the development of microbiology was directed by its own internal logic, along paths very largely separate from those followed by general biology.

The dominant interests of post-Pasteurian microbiology were the detailed analysis of the chemical activities of microorganisms, and of their roles in the turn-over of matter in the biosphere; the characterization of the microbial agents of infectious disease (which led in due course to the discovery of a new class of biological objects, the viruses), and the analysis of the interactions between agents of infectious disease and their animal or plant hosts. Exploration of the latter problem was responsible for the creation of a new discipline of functional biology, immunology. The phenomena of immunity are displayed only by animals of the vertebrate evolutionary line, and immunology is, in consequence, an area of functional biology which has no organic connection with microbiology. The historical link between the two disciplines was purely accidental, and stemmed from the fact that the basic phenomena of immunity were first recognized in vertebrate animals after an immune response had been elicited by microbial infection.

The first links between microbiology and general biology were forged at the start of the twentieth century, during the development of the new functional discipline of biochemistry. The discovery of alcoholic fermentation by cell-free preparations of yeast provided an experimental system for the chemical analysis of energy-yielding metabolic processes. Parallel studies on the mechanisms of alcoholic fermentation by yeast and of lactic fermentation by muscle tissues eventually led to the recognition of their far-reaching chemical homologies. A few years later, the analysis of the nutritional requirements of animals and microorganisms revealed a second unanticipated common denominator:

the 'vitamins', trace nutrients for animals, were shown to be chemically identical with the 'growth factors' which are trace nutrients for many microorganisms. The analysis of the metabolic role of these compounds – largely conducted, for reasons of experimental convenience, with microorganisms – showed that each vitamin is the biosynthetic precursor of a specific type of coenzyme, and that coenzymes have universal functions in cellular metabolism. Several metabolic homologies shared by all cellular organisms were thus revealed. During the 1930s, this new biological insight was epitomized in the phrase: 'the unity of biochemistry'.

However, microbiology made no contribution to the second major advance of biology in the early twentieth century: the creation of genetics. This science arose through a convergence between the Mendelian analysis of inheritance in plants and animals, and the discipline of cytology, which revealed the chromosome to be the physical vehicle of eukaryotic hereditary determinants. What little was then known about inheritance in microorganisms suggested that the laws of Mendelian inheritance did not operate at this biological level. The separation between microbiology and genetics ended only after 1940. The first junction was the isolation in 1941 by Beadle and Tatum of a series of auxotrophic mutants in the ascomycete *Neurospora*, coupled with the demonstration that the inheritance of these biochemically definable mutant traits obeys Mendelian laws. In 1943, Delbrück and Luria established a methodology (the fluctuation test) which showed that bacterial mutations are spontaneous random events, and not (as had previously been widely believed) subject to environmental determinism. This laid to rest the Lamarckian heresy, which had found a last foothold among bacteriologists. Two discoveries then opened the way to a rapid development of bacterial genetics: the demonstration of conjugation in *Escherichia coli* and of transformation in the pneumococcus. Although initially its significance was not widely recognized, the identification of the transforming principle as DNA by Avery and his collaborators first revealed the chemical nature of the gene.

During the decade between 1940 and 1950, the convergence between genetics, microbiology, and biochemistry set the scene for the second major revolution in the history of biology. Because of their relative simplicity, the bacteria and their viruses provided the principal experimental material for the elucidation of the laws of molecular biology. A century after Darwin, microbiology at last took its place in the conceptual framework of general biology.

V. Concluding Remarks

The attempt to define microbiology proves, like many problems of definition both in science and in other intellectual domains, to be a task

of great complexity and difficulty. One could of course sidestep the difficulties, by an arbitrary declaration that it is the branch of biology devoted to the study of small forms of life; and , indeed, no better one-line definition can be proposed. A comparable problem faced the writer E. M. Forster when he was asked to lecture on the Novel, and this essay can be appropriately concluded by quoting his response to the definitional challenge.

> 'Perhaps we ought to define what a novel is before starting. This will not take a second. M. Abel Chevalley has, in his brilliant little manual (*Le Roman Anglais de Notre Temps*), provided a definition, and if a French critic cannot define the English novel, who can? It is, he says, "a fiction in prose of a certain extent". That is quite good enough for us, and we may perhaps go so far as to add that the extent should not be less than 50,000 words. . . . If this seems to you unphilosophical will you think of an alternative definition, which will include *The Pilgrim's Progress, Marius the Epicurean, The Adventures of a Younger Son, The Magic Flute, The Journal of the Plague, Zuleika Dobson, Rasselas, Ulysses* and *Green Mansion,* or else will give reasons for their exclusion?'
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> *Aspects of the Novel* (Edward Arnold, London, 1927).

VI. Further Reading

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