From the Department of Bacteriology, University of California, Berkeley,
and the Hopkins Marine Station of Stanford University, Pacific Grove, California

The Concept of a Bacterium *

By

R. Y. STANIER and C. B. VAN NIEL

Since the earliest days of microbiology, the biological nature and
relationships of the bacteria have been subjects of perennial discussion.
Why have these questions obsessed some members of each succeeding
generation of microbiologists? There can be no doubt about the principal
reason. Any good biologist finds it intellectually distressing to devote his
life to the study of a group that cannot be readily and satisfactorily
defined in biological terms; and the abiding intellectual scandal of
bacteriology has been the absence of a clear concept of a bacterium.

Our first joint attempt to deal with this problem was made 20 years
ago (STANIER and VAN NIEL 1941). At that time, our answer was framed
in an elaborate taxonomic proposal, which neither of us cares any longer
to defend. But even though we have become sceptical about the value of
developing formal taxonomic systems for bacteria (see VAN NIEL 1946,
for an exposition of the reasons), the problem of defining these organisms
as a group in terms of their biological organization is clearly still of great
importance, and remains to be solved. A great deal of relevant infor-
mation has emerged in recent years, and the time therefore seems oppor-
tune for a fresh analysis of this question.

It is not our intention to review here the development of ideas about
the nature and relationships of the bacteria; readers who are interested in
the historical aspects of the problem can find authoritative and very full
accounts in PRINGSHEIM (1923, 1949) and VAN NIEL (1955).

The bacteria and their external affinities

The recognition and establishment of the bacteria as a distinctive
group of microorganisms was largely the work of FERDINAND COHN (1854,
1872, 1875). The type concept of a bacterium which emerged from the
studies of COHN was based primarily on the properties of a relatively

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* Since Professor E. G. PRINGSHEIM has made such great contributions to
knowledge of the bacteria as a biological group throughout his long scientific career,
we are happy to be able to dedicate this essay to him, as a token of our respect
and affection on the occasion of his 80th birthday.

homogeneous group: the unicellular eubacteria which multiply by binary fission. But as the exploration of the microbial world proceeded, other groups, whose properties differed to a greater or lesser degree from those of the classical unicellular eubacteria, came to be accepted by the biologists as "bacteria". These groups included the actinomycetes, the myxobacteria, the photosynthetic bacteria, the spirochetes, the rickettsias and the pleuropneumonia group, to mention only major accretions. The present diversity of the bacteria *sensu lato* is shown most strikingly by a simple enumeration of the biological properties which can exist among them. They can be photosynthetic or non-photosynthetic; motile by any one of three different mechanisms or permanently immotile; unicellular, multicellular or coenocytic; multiplying by binary transverse fission, by budding, or by the formation of conidia or gonidia.

The most remarkable feature of this extraordinary situation is that there has been so very little argument about the assignment of any particular organism or group of organisms to the bacteria. For example, the similarities in organismal construction between actinomycetes and mycelial fungi have only rarely led to disputes between the bacteriologists and the mycologists concerning the correct assignment of any microorganism that possesses this kind of construction. This example — and many others might be cited — shows that it is not, in general, difficult to distinguish a bacterium *sensu lato* from another kind of microorganism, even when there are considerable similarities of size and gross structure[1]. We have, therefore, a solid pragmatic basis for the belief that a scientific definition of the bacteria constitutes an attainable goal.

Let us consider what a scientific definition of the bacteria might be expected to accomplish. The bacteria include some forms, such as the rickettsias and the agents of psittacosis and trachoma, that are obligate intracellular parasites, and whose structural units are barely resolvable by use of the light microscope. Consequently, it is necessary to find criteria by which such forms can be distinguished from large viruses. On the other hand, the bacteria include many organisms which, in view of their size and organismal construction, might be confused with representatives of other protistan groups: algae, protozoa or fungi. It therefore follows that a definition, in order to be useful, should permit a clear separation of the bacteria *sensu lato* both from viruses and from other protists.

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[1] We cannot resist the temptation to cite a very striking specific illustration of this contention. About 30 years ago, BENEDEK (1927) described an eubacterium of large size, *Bacillus megaterium*, as a new fission yeast, *Schizosaccharomyces hominis.* This erroneous taxonomic conclusion was soon corrected by DORREPAAL (1930) who, as her paper makes clear, immediately recognized the organism as a bacterium upon simple microscopic examination of BENEDEK's culture.

The belief that the small, obligately parasitic bacteria of the rickettsia type are "transitional" between other bacteria and viruses used to be frequently expressed in the bacteriological literature (see, e.g. WILSON and MILES 1955). This belief could be held in the past, only because the essential nature of the viruses had not been clearly established. During the past 15 years the study of viral development, initiated through work with the bacteriophages and later extended to plant and animal viruses, has provided a detailed knowledge of the unique properties of this class of biological objects. The infectious particle, or virion, of a virus contains only one kind of nucleic acid (either RNA or DNA), which is enclosed in a coat of protein, or capsid, formed by the polymerization of identical protein sub-units, or capsomeres (LWOFF, ANDERSON, and JACOB 1959). The virion carries few, if any, proteins endowed with enzymatic function; and if such proteins are present, their role is specifically concerned with attachment to and penetration into the host cell. The virion cannot divide. During its replication, which occurs within a susceptible cell, the only component of the virion that is directly reproduced is its nucleic acid. As first explicitly stated in a classical paper by LWOFF (1957) published only 4 years ago, this constellation of properties defines a special kind of biological entity, *the virus*, which is wholly different from the more familiar kind of biological entity, *the cell*. The cell always contains both RNA and DNA. It always contains a large array of different proteins endowed with enzymatic function, which are in the main concerned with the generation of ATP and the synthesis of the varied organic constituents of the cell from chemical compounds present in the environment. The reproduction of the cell is characteristically preceded by an orderly increase in the amount of all its chemical constituents, and takes place by division.

No biological entity which could properly be described as transitional between a virus and a cellular organism is known at present; and the differences between those two classes are of such a nature that it is indeed difficult to visualize any kind of intermediate organization.

The biological objects accepted as bacteria clearly have a cellular organization as this has been defined above. We shall therefore adopt, as the first part of our definition, the statement that *all bacteria are cellular entities*. This premise in principle provides us with a ready means of allocating any particular obligately parasitic biological entity of small dimensions either to the bacteria or to the viruses. If one is unable to make a definitive assignment in any particular instance, this can now only be because the essential biological properties of the object in question have not been ascertained. All the information now available suggests that the agents of the rickettsioses, of psittacosis and of trachoma have a cellular organization and mode of reproduction;

they should therefore be considered as members of the bacteria *sensu lato*, not as viruses.

While the definition of bacteria as cellular entities suffices to distinguish them from the viruses, it does not distinguish them from other protists. It follows logically that if one seeks to define bacteria in a way that will distinguish them from other protists, the statement that they are cellular organisms must be supplemented by an enumeration of specific properties that are distinctive for the bacterial cell.

In affirming that microbiologists do not experience difficulties in distinguishing bacteria from other kinds of protists, we stated a general rule which has a significant exception. As first emphasized by FERDINAND COHN, there are close structural similarities between certain bacteria and certain blue-green algae. Despite this, it at first appeared possible to distinguish the two groups on a physiological basis, by defining the blue-green algae in terms of their photosynthetic metabolism and characteristic pigment system. Difficulties arose as a result of WINOGRADSKY's studies (1888) on the sulfur bacteria *Beggiatoa* and *Thiothrix* which, though structurally indistinguishable from filamentous blue-green algae, lack the characteristic photosynthetic pigment system of the group. In recent years, a considerable number of other filamentous, gliding organisms which lack a photosynthetic apparatus have been recognized (SORIANO 1947; PRINGSHEIM 1951; HAROLD and STANIER 1955; PRINGSHEIM 1957). Evolutionarily speaking, there can be little doubt that all these microorganisms, commonly regarded as bacteria, are in fact apochlorotic blue-green algae (PRINGSHEIM 1923, 1949; COSTERON, MURRAY and ROBINOW 1961). These examples show that, unless we wish to use physiological and biochemical instead of cytological criteria, we cannot in the last analysis separate the bacteria *sensu lato* from the blue-green algae. It therefore follows that any distinctive structural properties which could be used to characterize bacteria would be shared by blue-green algae, so that one could not formulate a definition of bacteria which would exclude these algae (STANIER and VAN NIEL 1941).

For a long time, biologists have intuitively recognized that the cell structure of bacteria and blue-green algae is different from that of other organisms, and should be characterized as "primitive"; but a satisfactory description of the difference has proved remarkably elusive. The revolutionary advances in our knowledge of cellular organization which have followed the introduction of new techniques during the past 15 years have changed this situation. It is now clear that among organisms there are two different organizational patterns of cells, which CHATTON (1937) called, with singular prescience, the eucaryotic and procaryotic type. *The distinctive property of bacteria and blue-green algae is the procaryotic nature of their cells.* It is on this basis that they can be clearly segregated

from all other protists (namely, other algae protozoa and fungi), which have eucaryotic cells. The remaining pages of this essay will be devoted to a discussion of the essential differences between these two cell types, upon which rests our only hope of more clearly formulating a "concept of a bacterium".

The organization of function in eucaryotic and procaryotic cells

The justification for using a single term, the *cell*, in describing the unit of structure of both eucaryotic and procaryotic organisms rests on the *equivalence of function* of these two kinds of entities. In each case, reproduction brings into play essentially the same series of characteristic events. Both kinds of cell are compatible with the same modes of infra-structure: unicellularity, multicellularity and the coenocytic state. Grossly considered as units of biochemical function, they are likewise equivalent: all modern biochemistry bears witness to this fact. 20 years ago, it was by no means evident that these two cell types were also genetically homo-logous; but today, the impressive body of evidence which has been furnished through the study of bacterial genetics gives us every reason to believe that they are. The differences between eucaryotic and procaryotic cells are not expressed in any gross features of cellular function; they reside rather in *differences with respect to the detailed organization of the cellular machinery*.

Within the enclosing cytoplasmic membrane of the eucaryotic cell, certain smaller structures, which house sub-units of cellular function, are themselves surrounded by individual membranes, interposing a barrier between them and other internal regions of the cell. In the procaryotic cell, there is no equivalent structural separation of major sub-units of cellular function; the cytoplasmic membrane itself is the only major bounding element which can be structurally defined.

This difference is most universally expressed in the organization of the nuclear region and of the enzymatic machinery responsible for respiration and photosynthesis. Whereas the nucleus of the eucaryotic cell in the interdivisional state is characteristically separated from the surrounding cytoplasm by a nuclear membrane, no such boundary appears to exist in the procaryotic cell. In the eucaryotic cell, the enzymatic machinery of respiration and of photosynthesis is housed in specific organelles enclosed by membranes, the mitochondrion and chloroplast, respectively. Homo-logous, membrane-bounded organelles responsible for the performance of these two metabolic functions have not been found in the procaryotic cell.

Despite the absence of clearly defined internal bounding structures, there is a separation within the procaryotic cell between specific regions that differ in function and in chemical composition. For example, cyto-chemical techniques define a discrete nuclear region, which is the unique

site of the cellular DNA; and electron micrographs of bacterial thin sections fixed by the technique of RYTER and KELLENBERGER (1958), while providing irrefutable evidence for the absence of a distinct nuclear membrane, show with particular clarity the very sharp separation between the nuclear and cytoplasmic regions. In such preparations, the nuclear region is evenly filled with very fine filaments, no doubt composed partly, if not entirely, of long DNA molecules, while the most conspicuous elements in the adjacent cytoplasm are the ribosomes. The structural distinction always remains perfectly clear; there is no intermingling of the two elements. Precisely how such a phase separation can be maintained without the interposition of a membrane remains an unsolved problem of cellular physical chemistry. It may be connected with the immobility of the cellular contents of bacteria and blue-green algae in the living state. Although the cytoplasm of some eucaryotic cells shows little if any movement, there are a host of phenomena, including ameboid movement, cytoplasmic streaming, the formation, migration and disappearance of vacuoles, the light-directed orientation of chloroplasts, and the migration of nuclei throughout fungal heterocaryons, which clearly attest to the widespread existence of internal mobility in the eucaryotic cell.

Peculiarities of nuclear and genetic organization in the procaryotic cell

The long uncertainty concerning the nuclear structure of bacteria and blue-green algae has been to a considerable extent dispelled during the past 20 years, following the introduction of satisfactory procedures for fixation and staining of these organisms, which we owe largely to ROBI-NOW (1944, 1945). During active growth, the procaryotic cell is characteristically multinucleate, a reflection of the fact that nuclear division runs somewhat ahead of cell division. The division of the nuclear elements, as revealed by classical cytological methods, involves a simple broadening and splitting of the nuclear material, without any fundamental change in gross structure during the divisional cycle (ROBINOW 1956). There is nothing in the divisional process which can be equated with a mitotic mechanism; and the DNA in each nuclear body seems to be associated with a single structural element. The recent studies of the nuclear structure of bacteria and blue-green algae by electron microscopy (e. g. RYTER and KELLENBERGER 1958; HOPWOOD and GLAUERT 1960a, b) simply confirm this inference which had been drawn from earlier classical cytological work.

We are probably still far from the time when it will be possible to construct a cytogenetics of procaryotic organisms; despite the impressive achievements of bacterial genetics, detailed genetic knowledge is still confined to a few species of bacteria, and the genetic study of the blue-green algae has not yet been begun. It seems worthwhile, nevertheless, to

see whether the findings of bacterial genetics can be in any way used to interpret the remarkably uniform and distinctive character of the procaryotic nucleus as revealed by cytological study. Much the largest body of relevant genetic information can be derived from the analysis of conjugation in *Escherichia coli*; the masterly study by WOLLMAN and JACOB (1959) on the mechanism of genetic transfer and the character of donor strains of this species has led to a very clear picture of its genetic organization. The first and most important finding is that all the genetic determinants of this bacterium are linearly arrayed in a single linkage group; if we apply classical cytogenetic terminology, we would therefore have to say that this bacterium has *a single chromosome*. The way in which genetic transfer takes place during mating has led to the assumption that the chromosome is normally circular, but can be opened at any point, such opening being an essential preliminary to the act of genetic transfer.

The genetic data accordingly suggest that nuclear division in *E. coli* involves the replication of a single, closed linkage group, followed by a separation of the two resulting genetic units. KELLENBERGER (1960) has recently made the first attempt to construct a model for such a nucleus and its replication. The picture suggested by the genetic findings is certainly not discordant with cytological evidence. If the genetic material in a procaryotic cell is confined to a single linkage group, the apparent absence of discrete, multiple, DNA-containing structures in the dividing nucleus becomes readily intelligible. Furthermore, the absence of a mitotic apparatus can also be understood. The essential function of a mitotic apparatus is to guarantee the equipartition of genetic material at the time of nuclear division when, as is universally the case in eucaryotic cells, the total body of genetic information is distributed over two or more distinct structural units, the chromosomes. But when all the genetic information is confined to a single element of structure, as in *E. coli*, the equipartition can in principle be achieved by far simpler means.

A second possible model for the procaryotic nucleus is provided by a highly specialized type of nucleus which occurs in the eucaryotic cells of ciliates, the macronucleus. The division of the macronucleus is amitotic; but this condition is fully compatible with genetic stability and survival, as shown by the natural occurrence of strains of ciliates which possess only a macronucleus. The secret of genetic stability appears to lie in the highly polyploid condition of the macronucleus. Thus, extreme polyploidy might also be invoked to explain the observed genetic stability of procaryotic organisms; but this possible interpretation is not compatible with the rates of spontaneous and induced mutation commonly observed in bacteria, which strongly suggest that most of these organisms are haploid.

The mechanisms of gene transfer and recombination so far discovered in bacteria all have certain common features which sharply distinguish them from sexual and parasexual recombination in eucaryotic organisms. Gene transfer in bacteria, whether by conjugation, transformation, or transduction, involves the introduction of a small fragment of the genome of a donor cell into a cell with a complete genome, except in rare cases of conjugation. The recipient cell thus does not become genetically equivalent to an eucaryotic zygote; it is a partial diploid or merozygote, in which haploidy is re-established by elimination of the supernumerary genes which do not get incorporated into the recombinant genome. Consequently, even that process of procaryotic gene transfer which involves cell-to-cell pairing—conjugation in coliform bacteria—is not *genetically* homologous with the sexual process as it exists in eucaryotic organisms; it does not give rise to reciprocal recombinants. The resemblances between a mating pair of coliform bacteria and a mating pair of eucaryotic gametes are, accordingly, superficial.

Cytoplasmic organization in eucaryotic and procaryotic cells

The differences between the cytoplasmic regions of eucaryotic and procaryotic cells are most strikingly apparent from the organization of the structural elements responsible for the performance of the two complex metabolic unit processes,respiration and photosynthesis.In eucaryotic cells, respiration and photosynthesis take place in specific membrane-bounded organelles or plastids, the mitochondria and chloroplasts respectively. Since these organelles can be separated from the rest of the cell as recognizable structures, their functional capacities can be directly determined.

The biochemical machinery of the chloroplast comprises the photosynthetic pigment system and associated enzymes of electron transport, required for the conversion of light energy into chemical bond energy. The chloroplasts of higher plants, the only ones so far studied in detail in terms of their enzymatic composition, also contain all the enzymes required for the conversion of CO_2 to the characteristic primary product of carbon assimilation, starch (ARNON 1955; WHATLEY, ALLEN, TREBST, and ARNON 1960). The fine structure of the chloroplast as revealed by electron microscopy of thin sections shows a basic homology in all eucaryotic phototrophs (GRANICK 1961). It is bounded by a double membrane, and contains a large number of elongated and flattened discs, having the gross appearance of paired lamellae, which lie in a region (the stroma) that is apparently devoid of fine structure. Recent evidence (PARK and PON 1961) suggests that the machinery of photosynthetic energy conversion (i.e., the pigment system and the electron transport system) resides specifically in the paired lamellae; the surrounding stroma is therefore probably the site of the associated biosynthetic enzymes.

The respiratory plastid, or mitochondrion, contains the machinery of electron transport responsible for the generation of ATP by oxidative phosphorylation, together with many enzymes involved in the terminal oxidation of organic substrates, notably the enzymes of the tricarboxylic acid cycle (GREEN 1960). The fine structure of the mitochondrion as revealed by electron microscopy is, like that of the chloroplast, fundamentally homologous in all eucaryotic cells (NOVIKOFF 1961). It is bounded by a double membrane, from whose inner layer arises a characteristic internal lamellar system, which differs both in dimensions and arrangement from the internal lamellae of the chloroplast. There is now good evidence to show that the internal mitochondrial membranes are the site of electron transport and oxidative phosphorylation; they also contain one enzyme of the TCA cycle, succinic dehydrogenase (GREEN 1960).

In sum, modern biochemical techniques have made it possible to define the specific metabolic processes that occur in mitochondria and chloroplasts, while modern cytological techniques have shown that these two types of organelles have distinct and characteristic fine structures. Furthermore, certain biochemical activities can be assigned to structurally recognizable internal regions in each kind of plastid. One further point deserves emphasis. While it was, of course, evident a priori that mitochondria, lacking the necessary pigment system, cannot perform photosynthetic reactions, the inability of chloroplasts to perform respiratory reactions was by no means self-evident. Direct analysis (ARNON, ALLEN and WHATLEY 1956) has shown that chloroplasts do not respire. In all eucaryotic phototrophs, the presence of mitochondria is therefore essential for respiratory function. As first indicated by the classical cytological studies of GUILLIERMOND (summarized in GUILLIERMOND, MANGENOT, and PLANTEFOL 1933), and now convincingly confirmed by electron microscopy, the photosynthetic cells and tissues of eucaryotic organisms in fact always contain both chloroplasts and mitochondria.

Procaryotic organisms also carry out photosynthesis and respiration. But in the procaryotic cell, these metabolic unit processes are performed by an apparatus which always shows a much smaller degree of specific organization. In fact, one can say that *no unit of structure smaller than the cell in its entirety is recognizable as the site of either metabolic unit process*. This statement can be made clearer by a detailed analysis.

Let us first consider the organization of the respiratory apparatus as it exists in the cells of such aerobic bacteria as *Bacillus megaterium* or *Sarcina lutea* (WEIBULL 1953 a, b; STORCK and WACHSMAN 1957; WEIBULL, BECKMAN, and BERGSTRÖM 1959; MATHEWS and SISTROM 1959)[1].

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[1] In these particular cases, a close analysis of functional localization can be made by virtue of the fact that the cell wall can be completely destroyed by lysozyme, and a controlled disintegration of the cell itself is therefore possible.

The machinery of electron transport is intimately associated with the cytoplasmic membrane, which also contains certain enzymes of the tricarboxylic acid cycle (e.g. succinoxidase). The membrane of the bacterial cell is thus *functionally* analogous to the internal membrane system of the mitochondrion. As a consequence of this organizational pattern, destruction of cellular integrity by rupture of the cytoplasmic membrane abolishes respiration: the soluble respiratory enzymes, located in the cytoplasm itself, flow out and thus become dissociated from the transport system of the membrane. The effects are entirely comparable to those which follow the osmotic or mechanical rupture of the isolated mitochondrion of the eucaryotic cell. Accordingly, in the context of respiratory function, the bacterial cell as a whole is the irreducible site of the metabolic unit process.

The absence of mitochondria in the cells of aerobic bacteria is confirmed by electron micrographs of thin sections, which do not reveal typical mitochondrial profiles. Such profiles are readily evident in thin sections of even very small and simple eucaryotic cells, for example those of yeasts (AGAR and DOUGLAS 1957). It should be mentioned, however, that membranous structures, which appear to be formed by the invagination and convolution of the cytoplasmic membrane, have been observed recently in thin sections of bacilli and actinomycetes (FITZ-JAMES 1960; GLAUERT and HOPWOOD 1960), characteristically in close association with sites of transverse membrane formation. These peculiar structures, which FITZ-JAMES has termed *mesosomes*, may represent localized centers of respiratory activity, but no definite evidence concerning their function has yet been adduced.

Let us now consider the structures associated with photosynthesis in the cells of blue-green algae and bacteria. Electron microscopic studies on blue-green algae (e.g. NIKLOWITZ and DREWS 1957; SHATKIN 1960) show that the entire cytoplasmic region is traversed by a complex system of paired lamellae, which appear to be structurally analogous to, and conceivably homologous with, the internal lamellae of the eucaryotic chloroplast, but are not separated from other regions of the cytoplasm by a common enclosing membrane. Fragments of this internal lamellar system can be isolated after breakage of the cell; they contain all the chlorophyll of the cell, and are endowed with photochemical function (PETRACK and LIPMANN 1961). They are thus functionally homologous with the paired internal lamellae of the chloroplast. The structural picture in purple bacteria is more varied. In most of the forms so far studied by electron microscopy, the cytoplasmic region appears to be packed with small spherical vesicles; the best evidence for their association with the photosynthetic process is provided by the observation that they are

absent from the cells of facultatively aerobic species which have been grown under aerobic conditions in the dark, and thus rendered essentially free of the photosynthetic pigment system (VATTER and WOLFE 1958). These vesicles are assumed to be identical with the submicroscopic pigmented particles, or chromatophores, which can be isolated after breakage of the cell (SCHACHMAN, PARDEE, and STANIER 1952) and which have been shown to catalyze the reactions of photosynthetic energy conversion (FRENKEL 1954). In one species, *Rhodospirillum molischianum*, electron microscopy has revealed that the cytoplasm contains lamellar structures, arranged in parallel layers of six to ten, which are remarkedly reminiscent of chloroplast grana. There is no sign of a bounding membrane separating such lamellar bundles from the surrounding cytoplasm (DREWS 1960). In summary, there is good evidence that the various lamellar and vesicular cytoplasmic structures observed in blue-green algae and purple bacteria are the sites of photosynthetic energy conversion; and the lamellar structures, at least, seem structurally homologous with the paired lamellae that are such a conspicuous internal feature of the chloroplast. But in the procaryotic cell these structures are not consolidated into a membrane-bounded organelle; accordingly, the contiguous cytoplasm contains the biosynthetic enzymes that, in the eucaryotic cell, are contained within the chloroplast. Also in the context of photosynthetic function, the whole procaryotic cell constitutes the irreducible site of the metabolic unit process.

In concluding this discussion, we should like to note one additional feature of respiratory and photosynthetic function in the procaryotic cell that is distinctive. Both in blue-green algae and in the facultatively aerobic purple bacteria, there is suggestive evidence for a very close *functional* linkage between photosynthesis and respiration; such a functional linkage cannot exist in the eucaryotic cell, owing to the fact that the two unit processes are carried on in different and spatially separated organelles. The existence of such a linkage was first indicated by the sensitivity of the respiration of purple bacteria to light: illumination drastically reduces, and sometimes almost totally abolishes, respiratory oxygen uptake (VAN NIEL 1941; JOHNSTON and BROWN 1954; CLAYTON 1955). The same phenomenon has been observed in blue-green algae (BROWN and WEBSTER 1953), but it has not been found in green algae (BROWN and WEIS 1959). This effect suggests that in the procaryotic cell the two electron transport chains of respiration and photosynthesis may contain enzymes that are common to both and that are not spatially separated. Further evidence for such a functional and structural linkage of the two metabolic processes is provided by the finding that in purple bacteria the succinoxidase system is associated with the chromatophores (COHEN-BAZIRE and KUNISAWA 1960).

Structures associated
with cell movement in eucaryotic and procaryotic organisms

Eucaryotic and procaryotic cells also differ with respect to the nature of the contractile organelles responsible for cellular movement in a liquid medium. 20 years ago, it would have appeared very difficult to make any useful generalizations about the nature of such contractile organelles in eucaryotic protists, since their gross structure, number, and position on the cell can vary so widely; at first sight, the various kinds of flagella found in the different groups of algae, protozoa and lower fungi, the undulating membranes of trypanosomes, and the very complex and highly organized ciliary apparatus of the ciliates do not share obvious common structural denominators. One of the great achievements of modern cytology has been the demonstration that all these organelles are constructed on the same fundamental pattern and probably share a common mode of origin. They invariably contain 11 fibrils, two of which lie in the center of the organelle, the other nine being disposed in a circle about two central ones. This multifibrillar system is surrounded by and enclosed in an extension of the cytoplasmic membrane. Within the cell, the outer ring of fibrils originates from a so-called basal body, which is homologous in structure with the centriole, and in some cases has been shown to arise by supernumerary divisions of this cellular entity (FAWCETT 1961).

Contractile locomotor organelles are also found in two major groups of procaryotic protists: the eubacteria and the spirochetes. Eubacterial flagella show a definite and remarkably uniform structure at all levels of resolution. A single bacterial flagellum, which can serve as the complete unit of locomotor function (as shown by the existence of motile, mono-flagellate bacteria) has the approximate dimensions of one of the internal fibrils of a eucaryotic flagellum. High resolution electron microscopy generally does not reveal any fine structure in bacterial flagella, although in a few eubacteria they appear to be made up from two or three finer fibrillar elements twisted helically about one another. Chemical analysis of isolated bacterial flagella shows that they consist of a single species of fibrous protein. This fact confirms an inference which can also be drawn from immunochemical and microscopic observations: namely, that bacterial flagella are not enclosed within the cytoplasmic membrane, and are chemically distinct from it (see WEIBULL 1960, for a general discussion).

The axial filament of spirochetes appears from recent electron microscopic studies to be structurally equivalent to a bundle of bacterial flagella (BRADFIELD and CATER 1952; SWAIN 1955, 1957). This set of fibrils is wrapped helically around the cell, and anchored in it at the two poles. Both bacterial flagella and axial filaments probably originate in the cytoplasm from basal granules; but these granules are far smaller

than the basal bodies of eucaryotic contractile locomotor organelles, with which they are not homologous.

Eucaryotic protists whose cells are not completely enclosed within a wall can move over solid surfaces by the process of directed cytoplasmic streaming known as ameboid movement. No procaryotic organism capable of such movement is known. However, another type of movement over solid surfaces which does not involve locomotor organelles occurs in many procaryotic protists. It is known as "gliding movement", and is characteristic of many blue-green algae and all myxobacteria. Certain specialized groups of eucaryotic protists (desmids, gregarines) have mechanisms of movement which can also be described as "gliding". However, in no case has the mechanism of such gliding movements been elucidated. Consequently, it cannot be decided at present whether the gliding movement of eucaryotic and procaryotic organisms is produced in the same way.

Structure of the wall in procaryotic cells

We have so far discussed the different organizational patterns in eucaryotic and procaryotic protists that serve for the performance of certain common cellular functions: the transmission of genetic material, respiration, photosynthesis and locomotion. In this section, we shall deal with a distinctive property of procaryotic organisms that is expressed in chemical, rather than cytological, terms: the composition of their cell walls.

The wall is a structure that appears to serve a purely mechanical function; namely, protection of the enclosed protoplast from physical— and particularly osmotic—damage. This function requires that the cell wall contain molecular entities that can be combined into a rigid envelope, with a tensile strength sufficient to counterbalance the turgor of the enclosed protoplast. In the various groups of eucaryotic protists, a variety of different polymeric substances (e.g., cellulose, hemicelluloses, chitin and silica) fulfill this requirement. In all procaryotic protists, the tensile strength of the wall appears to be primarily determined by another unique polymeric substance.

Studies on the chemical composition of procaryotic cell walls were initiated only some 10 years ago, following the development of satisfactory methods for their isolation and purification (SALTON and HORNE 1951). This work was begun with eubacteria, and most of our information about the wall composition of procaryotic organims is restricted to this group. Within the eubacteria, there are considerable variations in the gross composition of the wall and in its chemical complexity. From the initial welter of chemical data, significant common features first emerged as a result of comparisons of the composition of the walls of Gram positive

eubacteria (Cummins 1956; Salton 1960a, b). It gradually became evident
that a common type of macromolecule is always responsible for the
rigidity of the wall in these eubacteria (see review by Work 1961). This
substance is a mucopeptide, which has a polysaccharide backbone con-
sisting of alternating residues of acetyglucosamine and acetylmuramic
acid. Attached to the backbone by peptide bonding with the carboxyl
groups of acetylmuramic acid are short chains of amino-acids (Salton
and Ghuysen 1960). These invariably include glutamic acid and alanine,
which occur at least in part as the unnatural D-isomers, and either L,L- or
meso-diaminopimelic acid, or lysine. In a few Gram positive bacteria, this
mucopeptide is the only constituent of the wall; in other Gram positive
bacteria, the wall also contains mucopolysacharides, and sometimes also
teichoic acids. Walls composed only of the mucopeptide are totally dis-
integrated, either in the isolated state or *in situ* on the cell, by treatment
with lysozyme, which specifically attacks the 1,4 glycosidic bonds linking
adjacent units of glucosamine and muramic acid. The well-known
osmotic lysis of many Gram positive bacteria which results from exposure
to lysozyme is hence attributable to the attack on the mucopeptide com-
ponent of their walls, and indicates the paramount importance of this
component in conferring rigidity on the wall as a whole.

The available analytical data indicate that the much more complex
walls of Gram negative bacteria also invariably contain the mucopeptide,
but here it is as a rule a relatively minor constituent of the whole wall
fabric (Salton 1960a, b). Gram negative bacteria as a group are not
susceptible to osmotic lysis as a result of treatment with lysozyme. Some
years ago it was shown, however, that such susceptibility can be induced
if they are simultaneously treated with versene (Repaske 1956). This
fact indicates that also in the walls of Gram negative bacteria, where it
generally represents a quantitatively minor constituent, the mucopeptide
is responsible for mechanical integrity; if it is made accessible to lysozyme
by versene treatment, its destruction entails a weakening of the wall suf-
ficient to permit osmotic lysis of the cell.

The importance of the mucopeptide for the mechanical properties of
the bacterial cell wall is also indicated by observations on the mode of
action of penicillin. As first shown by Lederberg (1956, 1957), the
penicillin-induced lysis of *Escherichia coli* can be prevented in an isotonic
medium; under such circumstances, the growing cells are converted to
osmotically sensitive spheroplasts, which undergo irreversible destruction
only if the medium is diluted. Independently, Park and Strominger
(1957) showed that during penicillin treatment *Staphylococcus aureus*
excretes uridine nucleotides of muramic acid, some of which also contain
the characteristic amino-acids of the mucopeptide. Penicillin accordingly
appears to inhibit the incorporation of the subunits of the mucopeptide

into the growing bacterial cell wall, so that death of the growing cell ensues as a result of osmotic lysis.

We must now consider the question whether the mucopeptide is an *exclusive and universal* component of the procaryotic cell wall. The two distinctive sub-units of the mucopeptide are muramic acid and diamino-pimelic acid. The former is always present in the mucopeptide, whereas the latter is sometimes replaced by lysine, which is a common component of proteins. Muramic acid is accordingly the best chemical indicator of the presence of the mucopeptide, but studies on its distribution outside the eubacteria have been confined to a relatively small number of organisms. It has never been found in eucaryotic cells, but has been identified in the walls of some myxobacteria, rickettsias and blue-green algae.

The natural distribution of diaminopimelic acid has been extensively surveyed by WORK (1951), WORK and DEWEY (1953) and HOARE and WORK (1957). Either the L,L- or the meso-isomer of this amino-acid is present in the hydrolysates of the cells of all bacteria and blue-green algae examined, with the exception of Gram positive cocci; later detailed studies on wall composition have shown that in this sub-group of eubac-teria diaminopimelic acid is replaced by lysine. On the other hand, WORK and DEWEY were unable to detect diaminopimelic acid in hydrolysates of higher algae, protozoa, fungi, and tissues of higher plants and animals. One exception to this rule subsequently emerged: FUJIWARA and AKABORI (1954) and HOARE and WORK (1957) detected traces of diaminopimelic acid in a green alga, *Chlorella ellipsoidea*. Since VOGEL (1959) has shown that *Chlorella*, like many bacteria, synthesizes lysine through a biosyn-thetic pathway in which diaminopimelic acid serves as a metabolic precursor, it is probable that the traces of diaminopimelic acid detected in hydrolysates of *C. ellipsoidea* reflect the presence of this amino-acid as an intermediary metabolite, and not as a structural constituent of the wall.

Perhaps the best present evidence for the absence of the mucopeptide as an essential structural element in eucaryotic cells is provided by the spectrum of action of penicillin, which is non-toxic for higher algae, protozoa, fungi, plants and animals. Despite the existing gaps in our knowledge, it thus seems probable that the existence of the mucopeptide in the wall constitutes a supplementary specific character for the defi-nition of the procaryotic cell.

A cell wall, although it protects the cell against osmotic shock, is not an absolutely indispensable element of cellular structure. In the case of the bacteria, the best evidence for this conclusion is provided by the elegant experiments of LEDERBERG (1957) on the behavior of *Escherichia coli* during growth in the presence of penicillin: provided that the medium is of such a nature that the cell is protected from osmotic shock, the

weakening of the wall structure does not affect the viability of the cells, which can grow as spheroplasts, and regain once more a normal form and wall structure if the penicillin is removed or destroyed. The question may therefore be asked whether there might not exist in nature procaryotic organisms which have lost *irreversibly* the capacity to synthesize the mucopeptide, but can nevertheless survive in the form of osmotically sensitive protoplasts or spheroplasts. The irreversible formation of such entities from normal eubacteria has in fact been repeatedly shown, for a large number of species, under laboratory conditions; these entities are the so-called "L-forms" of bacteria (KLIENEBERGER-NOBEL 1960; KANDLER and KANDLER 1960). The most effective general method to obtain them is the cultivation of bacteria in the presence of penicillin, in a medium with an elevated osmotic pressure. Under such conditions a bacterial culture may either become converted to spheroplasts which, as in the experiments of LEDERBERG, are capable of immediately reverting to the original state after penicillin removal, or give rise to L-forms. The factors which lead to a genetically stable L-state are not yet clear; but there is evidence to suggest that it is not simply a question of the selection of mutants having a deficient capacity for wall synthesis (LANDMAN and GINOZA 1961). Analytical studies by KANDLER and ZEHENDER (1957), and SHARP (1960) have shown that certain stable L-forms do not contain diaminopimelic acid and hence probably do not synthesize the muco-peptide. Its absence would explain both the osmotic sensitivity of these objects, and their strange structure, which resembles so little the struc-ture of the bacteria from which they have been derived.

The L-forms are human artifacts; but much evidence suggests that analogous forms can arise in nature and are able, in suitable environments, to multiply indefinitely in the L-state. These are the pleuro-pneumonia-like organisms (PPLO group), which can be most readily interpreted in biological terms as bacteria which have lost the ability to form the muco-peptide, and have subsequently become adapted to existence in natural environments where the absence of a mechanically strong cell wall is not a lethal character. The absence of mucopeptide components has in fact been demonstrated for a few strains belonging to the PPLO group (KANDLER and ZEHENDER 1957); if this is true of the group as a whole, these forms constitute one (and probably the only) procaryotic assem-blage lacking this otherwise distinctive feature of the procaryotic cell.

Epilogue

In their totality, the bacteria cannot be clearly separated from an-other large microbial group, the blue-green algae. Both groups have a cellular organization, designated as procaryotic, which does not occur elsewhere in the living world. The principal distinguishing features of the

procaryotic cell are: 1. absence of internal membranes which separate the resting nucleus from the cytoplasm, and isolate the enzymatic machinery of photosynthesis and of respiration in specific organelles; 2. nuclear division by fission, not by mitosis, a character possibly related to the presence of a single structure which carries all the genetic information of the cell; and 3. the presence of a cell wall which contains a specific mucopeptide as its strengthening element.

As PRINGSHEIM (1949) has so persuasively argued, the bacteria and blue-green algae encompass a number of distinct major groups, which do not now appear to be closely related to one another; their only common character is that they are procaryotic. It thus appears that the procaryotic cell has provided a structural framework for the evolutionary development of a wide variety of microorganisms. The evolutionary diversification of the procaryotic protists is expressed in: 1. gross organization, leading to the existence of unicellular, multicellular and coenocytic groups; 2. mode of cellular locomotion; 3. mode of cell division; and 4. major patterns of energy-yielding metabolism, evidenced by the existence of three entirely distinct groups of phototrophs (blue-green algae, purple bacteria and green bacteria), as well as an unrivaled range of specialized chemotrophic groups. With respect to all these features, there are parallel modes of evolutionary diversification among the eucaryotic protists (i.e. other groups of algae, protozoa and fungi). Consequently, if we look at the microbial world in its entirety, we can now see that evolutionary diversification through time has taken place on two distinct levels of cellular organization, each of which embodied, within certain limits, the same kinds of evolutionary potentialities. Only the eucaryotic cell appears, however, to have contained the potentialities for the development of highly differentiated multicellular biological systems, and accordingly only this kind of cell was perpetuated in the evolutionary lines which eventually gave rise to higher plants and animals.

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Professor R. Y. STANIER
Department of Bacteriology, University of California, Berkeley, California/USA