THE EVOLUTION AND RELATIONSHIP OF THE GREAT GROUPS OF BACTERIA

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It is a difficult feat of the imagination to reconstruct the path of evolution of any group of organisms, especially that of the bacteria. In all cases certain difficulties are encountered; but, whereas among the higher organisms one is troubled by the lack of transitional types, the main difficulty among bacteria lies in the superabundance of intermediate strains. Moreover, while, among the former, one is guided chiefly by gross structural distinctions, among the latter, one has to rely to a great extent on the finer biochemical and metabolic differences. Yet an attempt to trace the evolution of these simple cells may well lead to a clearer conception of the character of the organisms and the nature of their adaptation to a saprophytic, parasitic or pathogenic mode of life.

Impossible as it may be to say with certainty when and how bacteria originated, the evidence, little as it is, seems to point to these minute unicellular organisms as among the most primitive of living forms. The simple structure of the cell and the absence of a formed nucleus mark them as possibly the simplest type of cell. The ability of some types to subsist on simple inorganic substances (CH₄, NH₃, and CO₂) without the aid of sunshine, and the sensitiveness of all bacteria to the action of sunlight suggest their existence on this planet prior to the appearance of plant life or the penetration of the rays of the sun.

1 For a full discussion of the subject see the article by Henry Fairfield Osborn on The Origin and Evolution of Life upon the Earth, Scientific Monthly, 3, 1916.
through the volcanic vapors. Finally, Walcott’s discovery of bacteria closely resembling our nitrogen fixers of the soil in Algonkian deposits is an added proof of the primitiveness of these microbes.

The intimate dependence of both plants and animals on bacteria and their activities tends to strengthen the conviction that these microorganisms must have preceded the others. That plants are directly dependent on bacteria is well known. Plants cannot subsist without nitrates and soluble phosphates and both these substances, unless provided as chemical fertilizers, are rendered available in the soil through the action of bacteria. In the arid regions where plant life is absent Lipman found that bacterial life was also absent and where plants do exist they send their rootlets into that part of the soil where bacterial activity is most abundant. That animals cannot develop in the absence of microbial life is well illustrated by the futile attempts to cultivate amoebae without the aid of living or dead bacteria. Recently this relationship was even more strikingly emphasized by the experiments conducted by Loeb who, though successful in hatching adult flies from sterile eggs in sterile media, has so far failed to carry them through the second generation on sterile fruit, although fruit is the normal food of the fly in question.

The story of the evolution of bacteria, like that of the higher forms, must be from the simple to the complex—but along a different line. Microbial evolution must be conceived as an adaptive development in the direction of mobilizing more and more molecules (enzymes) to enable the cell to utilize the more and more complex nitrogen and energy-yielding substances which accumulate in the soil as a result of their own and other biotic activity. It is the probable course of these adaptive modifications that I shall attempt to trace.

It is well known that the smaller the body the greater, proportionately, are its energy requirements. Bacteria need only minute amounts of nitrogenous food but require a relatively enormous quantity of energy-yielding (carbon) compounds. It is, therefore, reasonable to expect the trend of bacterial evolution to have been in the direction of the utilization of a
wider range of energy-yielding substances. We know that most saprophytic bacteria (and even many of the parasitic forms) when supplied with a suitable carbon compound—usually glucose—can utilize either $\text{NH}_3$ or $\text{NO}_3$ nitrogen. At the same time we find great diversity among them in their ability to use various carbon compounds. It is not merely an accident that bacteriologists have in recent years turned to carbohydrates and related substances for the differentiation of bacterial types. It is in this direction that greatest differentiation exists and this is most probably the path of early evolution.

With the increasing deposits of decaying nitrogenous matter a new line of adaptation began. Accordingly we find that as we go higher in the scale of bacterial life the power of utilizing complex nitrogenous compounds (the amino-acids, the proteoses and even simple and complex proteins) comes more and more into play.

The probability that the course of bacterial development was as suggested is strengthened by other evidence. The sparing effect that carbohydrates, (Kendall) alcohols (and, in all probability, other energy-yielding substances) exert on the utilization of nitrogenous compounds, or, in other words, the preference shown by bacteria for carbonaceous food; the inhibitive influence that sugars have on the secretion of proteolytic enzymes (Kligler, Kendall and Walker); the preference shown by certain types of bacteria for the simpler carbohydrates as shown in the metabolic gradients, both among the colon-typhoid group and the cocci; the ability of a large number of bacteria to utilize a wide range of energy-yielding substances from formic through the other fatty acids, the triatomic alcohol and more complex sugars and alcohols; the ability of a considerable number of common bacteria to obtain their nitrogen readily from ammonia; and, finally, the inability of many of the common bacteria to utilize proteoses and complex proteins, though they can use simple amino-acids—all indicate an earlier and more deep-seated development of fermentative powers, followed by later adaptation in the direction of the elaboration of pepto- and proteolytic enzymes.

This evolutionary process is observed in both group and
species development. The colon-typhoid bacteria and the streptococci form distinctive fermentative groups, while the aerobic and most of the anaerobic spore-bearing bacteria constitute distinctive proteolytic groups. At the same time there are members of the former groups that secrete proteolytic enzymes (B. cloacae, proteus forms, staphylococci, micrococci) while some of the aerobic and practically all the anaerobic spore-formers are capable of at least fermenting glucose, which, as is the case in the fermentative group, exerts a definite sparing effect on the secretion of proteolytic enzymes. The dominant and subordinate activities of these two groups indicate clearly, however, the sequence of their development.

Nor are these functional changes entirely unaccompanied by structural modifications. In some cases, to be sure, such differences are not discernible; in others, however, they are more marked. These structural differences are brought out in the behavior of the cells to stains—the ability of the cell to bind iodine, due, probably, to variations in the lipoidal content; the presence of definite granular (chromatin) material; the presence of branching and, finally, the presence of spores. The simple fermentative types are gram negative, show no granular structure and bear no spores. The diphtheria and tuberculosis groups are gram positive, have a definite granular structure, possess little fermentative power but definite proteolytic activity, the nature of which is not yet known. Finally, we have the usually gram positive spore-bearing bacteria with distinct proteolytic tendencies.

In the course of their evolution, with the advent of plant and animal life, some of these micro-organisms have become adapted to a parasitic existence often to the detriment of their host. With this adaptation to the host there followed, usually, a loss or modification of function but a general relationship and resemblance to saprophytic ancestors remained. As a result we find in almost every group of bacteria (as in the case of other parasitic plants and animals) both parasitic and pathogenic, as well as saprophytic forms. In all these cases the presumption naturally is that the parasitic and pathogenic types are off-
shoots from the saprophytes and that the main line of evolution was carried on by the saprophytic bacteria in response to a particular selective environment.\(^2\)

Glancing over the range of microbial life we find purely oxidative processes associated with the simplest and probably the most primitive bacteria—the prototrophic forms. These organisms derive their food from simple inorganic substances, the oxidation of C, N, S and Fe compounds furnishing the energy. It is not at all improbable that the four groups of oxidizers—the carbon, sulphur, iron and nitrogen oxidizers, respectively—arose at about the same time, independently of one another. Nevertheless it seems fairly certain from the important part played by carbon compounds in the vital activities of our common bacteria, especially as a source of energy, that the carbon oxidizers are the forerunners of the bacteria of today. Starting, therefore, with methane, the simplest carbon compound, at the base line, the oxidizers of CO would follow and from them would arise in succession those organisms capable of utilizing CO\(_2\), formic acid, acetic acid, alcohol, etc. Since the ammonia and nitrite oxidizers (or nitrifiers) also assimilate large amounts of carbon-dioxide, (Jensen) they would seem to fall in line along with those organisms capable of obtaining their energy from carbon-dioxide.

\(^1\) Whether these selective influences were exerted on slight cumulative variations or on mutations does not affect the general argument. Examples of what may appear to represent one or the other mode of development exist. The slow transition from the aerogenes type through the \(B.\) \(cloacae\) to \(B.\) \(proteus\) may be an instance of gradual selection, while the abrupt transition from the non-spore-bearing to spore-bearing bacteria may well be cited as an instance of mutation. Fluctuating variations are abundant among bacteria due to the simple character of the cell and its intimate relation to the environment which renders it highly susceptible to external influences. As yet, true mutations have been definitely shown to occur only in a few isolated instances, where single cell cultures were used. But even in these cases the possibility of gradual selection cannot be excluded, since the newly acquired property (usually the power to ferment a given carbohydrate) manifests itself only after the colony is a few days old, in other words, after numerous generations have arisen from the single parent. In connection with the general thesis it is interesting to note that the new character acquired by these mutants is usually one already existing in some ancestral type of the group.
All these bacteria are strictly aerobic, depending on the oxygen of the atmosphere for their oxidative processes. At about this stage of evolution, however, with the accumulation of stable oxides, a new branch developed, differing from the main trunk in its power of utilizing combined oxygen for intracellular combustion. The prototrophic denitrifying bacteria described by various authors are most probably the progenitors of this group. This radical deviation from type gave rise to a new and distinct line. On the one hand we have the strictly aerobic, oxidative, usually gram positive, non-fermentative organisms with proteolytic tendencies and a complex cell structure—the nitrogen fixers (possibly the fluorescent forms fall in here), the diphtheria, tuberculosis and actinomyces groups; on the other hand we have the facultative aerobic, reducing (i.e., obtaining their oxygen from an oxy-compound), usually gram negative strongly fermentative simple cells—the aerogenes, colon, typhoid, proteus, septicemia groups. The striking and fundamental morphological, chemical and immunological differences existing between these two groups leave no doubt that they represent two distinct lines of development. Moreover the relative stability and definiteness of type characterizing the first group are indicative of a more remote origin while the relative instability and the large number of transitional forms found in the second group point to a more recent development. Recency of origin and instability of type run a remarkably parallel course.3

The line of descent from the prototrophic denitrifiers is entirely clear. The capsulated aerogenes group has in the last two years been definitely shown to be a saprophytic soil and grain type. Its relation to the so-called prototrophic denitrifiers, though not altogether established, is borne out by its power to live in simple inorganic media and under certain conditions, even to fix atmospheric nitrogen (Löhnis). With more detailed study of soil bacteria it may even be possible to find intermediate forms. From this type two groups arise—one essentially parasitic, the other saprophytic. The parasitic

1 The tuberculosis and dysentery groups respectively are examples of the two classes.
branch starts with the fermentative gas-producing \textit{B. coli} types, which lose their fermentative character with increasing parasitism and develop intermediate forms ranging from the fermenters with gas through fermenters without gas to non-fermenting types. This leads through the typhoid and dysentery bacilli to the septicemia group.

The second or saprophytic branch evolves by way of \textit{B. cloacae} (almost identical with \textit{B. aerogenes}, except for lack of capsule formation and the added variable power to secrete a tryptic enzyme) to the proteus group, which possesses more intense proteolytic properties developed at the expense of the fermentative powers. The \textit{B. vulgaris} still actively ferments glucose and sucrose though, like the paratyphoid, it has lost the power to ferment lactose. Being saprophytic in character, however, it has developed the property (first manifested by the \textit{B. cloacae}) of actively secreting a tryptic enzyme. This is manifestly the first type possessing both marked fermentative and proteolytic characters. From this type two lines of development are possible,—one, leading to the complete loss of fermentative powers (which appears to be the tendency in the whole group) and consequently a condition of more or less strict aerobiosis; the other, retaining both characters but undergoing other modifications.

These two lines are represented by the aerobic and anaerobic spore-bearing bacilli, respectively. Just how and when spores evolved is, of course, impossible to say but there are many indications of the relationship between the spore-formers (aerobic as well as anaerobic) and the \textit{B. proteus}. The relationship with the former is shown by the existence of proteus-like, non-fermenting, strictly aerobic but, as yet, non-spore-forming bacilli which have been repeatedly reported by various authors. These are all soil forms. The kinship with the anaerobes is indicated by the constant association of the proteus bacilli with putrefactive processes; the inability on the part of the anaerobes (with the exception of \textit{B. welchii}) to ferment lactose while still retaining the power to ferment glucose and to some extent sucrose; and, finally, by the marked acid hydrolysis of pro-
teins manifested by both groups of organisms. The transition from facultative aerobiosis to obligatory anaerobiosis is not at all unusual among bacteria. Different degrees of sensi-
tiveness to oxygen tension are manifested by closely related organisms in different groups (e.g., the cocci, the diphtheroids), and it is readily conceivable that an organism subjected to prolonged existence under anaerobic conditions would develop a high degree of sensitiveness even to traces of free oxygen.

A third group of bacilli, which probably had its origin in the proteus family, is that of the pigmented bacteria, including *B. prodigiosus*, *B. rubrum*, etc. Like the proteus forms they are gram negative, peritrichic, liquefying bacteria, some of them also producing gas from glucose. Pigmentation is often found among the cloacae forms and in our collection there are yellow and red pigment producers isolated from water, both of which otherwise behave like typical *B. coli*.

Returning again to the aerogenes group one finds many striking resemblances between these organisms and the capsulated chain-forming cocci. The similarity in the character of the capsule (solubility in acetic acid); the lanceolate shape of the cell; the power of both to ferment inulin; their localization in the same organs causing similar disturbances; their active fermentative properties point to a rather close relationship. The fact that the chain bacteria are gram positive indicates a radical structural modification analogous to that in the spore-forming bacteria. Here, again, a comparatively recent development is indicated by the marked instability of types. The non-cap-
sulated streptococci are obviously related to the pneumococci while the milk streptococci are but little removed from the aciduric lactic acid bacilli, usually found in milk and milk products.

While the course of the development of this large branch, composed of the so-called reducing bacteria can be traced with a fair degree of certainty, the path of descent of the main trunk consisting of the strongly aerobic and more complex forms is not quite as clear. The uncertainty regarding the latter group is attributable to at least two factors. It is due partly to the
fact that the metabolic activities of these organisms have not been studied as carefully and partly to the fact that, being the older and more stable group, many of the linking intermediate forms have disappeared and only the dominant types have remained. But even though the details remain somewhat obscure the general relationship is very clearly indicated. The saprophytes from which our second main stem arose are the nitrogen fixers of the soil (azotobacter), powerful oxidizers, oxidizing nitrogen to nitrates and ethyl alcohol to acetic acid (Jensen). Their nearest relative is the nitrogen fixer of the legumes which may really be considered a plant parasite localizing in the root of the plant and producing a reaction similar to that caused by the tubercle bacillus in the resistant animal body. As a parallel offshot to the B. radicicola we have the saprophytic acid fast bacteria (associated usually with grass), and their parasitic relatives, the various types of the tubercle bacillus. Recently the relationship between the acid fast forms and the actinomyces has been clearly established (Claypole). In this latter group, also, there are the soil saprophytes, associated with grass digestion (Conn), as well as human and animal parasites. Arising somewhere from the acid fast group there is the specific parasitic diphtheroid group which has been found nowhere (as far as I know) outside of the animal body. As parasites these are widely distributed in animal tissues, though, up to the present, only the diphtheria bacillus has been found to be definitely pathogenic. The relation of the diphtheria bacillus to the sporothrix is suggested by transitional forms found in the human mouth (Kligler). These organisms are highly pleomorphic showing thread, coccoidal and diphtheroid forms. They are fermentative, aerobic types. The oral cavity seems to be a favorite locality for the whole diphtheroid group.4

While there is an apparent gap between the azotobacter and the acid fast bacilli, there are still many points of similarity. The acids fasts can thrive on a simple medium with ammonium

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4 This localization may account for the fact that this group differs so markedly from the other members of the class in its power to ferment carbohydrates, particularly glucose and maltose.
compounds as a source of nitrogen and glucose as a source of energy, while recent investigation has brought out the fact that the azotobacter can assimilate free nitrogen more readily if glucose and a small amount of ammonia are supplied. The nodule nitrogen fixers with their branching cells and localized infective power with nodule formation show even more marked resemblances to the acid fast bacteria. The whole group is characterized by absence of fermentative properties (except the diphtheroids), strict aerobiosis, a peculiar nitrogen metabolism the nature of which is not at all clear (this applies to all the members of the group), the gram positive reaction, a complex granular structure of the cell with a tendency towards branching and finally the peculiar local reaction often nodular in character which is produced by the pathogenic varieties.

The general parasitic character of the white staphyloccoci, their moderate fermentative character, lack of reducing and liquefying properties, together with their gram positive reaction and the diphtheroid character of their growth link them to the diphtheroid bacilli. From white cocci there arise the more highly parasitic and pathogenic staphyloccoci on the one hand and the saprophytic micrococci on the other. A group of transitional saprophytic albococci has been shown to exist (Winslow), while yellow pigmentation is often observed among the diphtheroids.

There is one group of organisms—the fluorescent forms—the position of which is hard to determine. We find soil nitrogen fixers that produce fluorescence. On the other hand the fluorescent bacteria are strong ammonifiers, though they, like the nitrogen fixers, are aerobic and non-fermenters. Their simple structure, lophotrichic flagella and gram negative reaction mark them as simple bacteria. Perhaps they occupy an intermediate position. For the present we may place them with the oxidative bacteria.

The vibrios are so closely akin to the fluorescent (or pseudomonas) bacteria that their place in the scheme outlined depends on the position assigned to their related group. In fondness for oxygen, general proteolytic character, and in structure and
appearance of cell these groups resemble each other so closely as to make it at times difficult to differentiate them from one another.

The accompanying diagram attempts to present in a schematic way by no means rigid or exact the probable lines of evolution of the different groups of bacteria. Much of it may be faulty and it is obviously incomplete, but in its essential outline it seems to me to be correct and helps to show these organisms in their probable relationship to one another as members of a single if diversified order.