SCIENCE

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THE CHARACTERIZATION AND CLASSIFICATION OF BACTERIAL TYPES[1]

THE vast majority of students of microbial life are preoccupied with immediately practical problems, and most of them have been trained for their work from the standpoint of some practical art, medicine, veterinary science, sanitary engineering or agriculture, rather than from the more general and fundamental standpoint of the biologist. The Society of American Bacteriologists was founded as a protest against such necessary but dangerous specialization, to bring together workers in all fields for a consideration of their problems in the light of the underlying, unifying principles of bacteriology as a member of the group of the biologic sciences. It is this ideal which distinguishes our society from any other organization in America which deals with microbic life and its effects.

It is of course fruitless to attempt to draw any sharp distinction between pure and applied science, and it would be a great pity if, as we gather year by year, we should fail to discuss together many of the more special problems of technique with which we are concerned. In particular it is well that we should exercise the widest hospitality toward those branches of our science, such as dairy bacteriology and soil bacteriology which have no technical societies at their disposal, such as are available for the specialists in medical and sanitary lines. We should be untrue to our highest mission, however, if we failed at the same time to emphasize those phases of our work in which this society of all others

[1] Presidential address, Society of American Bacteriologists, Montreal, Canada, January 1, 1914.

is peculiarly qualified to be of service, in striking sparks by the contact of experience in the different fields of bacteriology, and in viewing all our special problems by the clear light of fundamental biological principles.

The task of the biologist is the study of the reactions of the group of allied substances we call protoplasms, under the influence of various physical and chemical conditions of the environment. Instead of the pure compounds of the chemist we must deal with organisms, interacting mixtures of substances, different for each kind and even for each individual plant and animal. In very refined work such as is involved in the determination of reaction times by the psychologist or in our own studies of the action of disinfectants, even the personal equation of the individual or the individual strain must be taken into account. For most purposes, however, the species or kind of organism displays reasonably uniform characteristics, and may be used as our practical unit of study. A clear distinction between the kinds of organisms involved and a clear conception of the relation between these kinds is certainly however imperative, and a sound basis for the characterization and classification of the organisms with which we deal is one of the most pressing needs of bacteriology.

The fact that we have lacked in the past any sound system of sorting out and arranging bacterial types requires no elaborate demonstration. The question of what constitutes a colon bacillus has agitated sanitary bacteriologists for three decades and is still unsolved. And to take a still more striking and still more important case, consider the controversy as to the Vielheit or the Einheit of the streptococci, which has raged so long. Here is a group of organisms, the part played by which in a wide range of diverse diseases is found to be more fundamental—a group which I am inclined to think produces in the aggregate more suffering and death—than any other group, except the acid-fast bacilli. Yet we are almost wholly at sea in regard to their identification and mutual relationships.

There are two very distinct types of variations characteristic of organisms in general, fluctuations and mutations, and both are well recognized among bacteria. Fluctuations are the minor quantitative differences which group themselves on a curve of frequency when a large series of individuals is studied, as a rule due to the chance effects of environment and not inheritable. Thus, for example, Walker and I (Winslow and Walker, 1909) found that one hundred different subcultures of a single strain of the paratyphoid bacillus race *A* gave acidities in dextrose broth varying between 1.1 and 1.6 per cent. normal, while a similar series of lines of race *B* gave acidities varying between 1.3 and 1.9 per cent. normal. We took the subcultures of each type giving maximum and minimum acidities and isolated one hundred secondary subcultures of each. The curves from these extreme cultures however in spite of the diversity exhibited by their immediate parent stock, went back to the curves characteristic of their respective races, with a mean value of 1.4 for race *A* and of 1.6 for race *B* (Fig. 1). These fluctuating variations are clearly of no systematic significance and must be eliminated from consideration by a study of numerous strains of the same type.

It seems on the whole most convenient to limit the term fluctuations to such non-inheritable variations as those just described. At times, however, we find in our cultures variations of apparently similar nature, but distinguished by the fact that selection among them does produce permanently different races. Thus, Goodman (1908) carried out experiments with the acid production of *B. diphtheriæ* somewhat

like those with the paratyphoid strains which have just been discussed, but leading to the ultimate separation of two strains of widely different fermentative power. Rettger and Sherrick (1911) report similar results in regard to the pig-

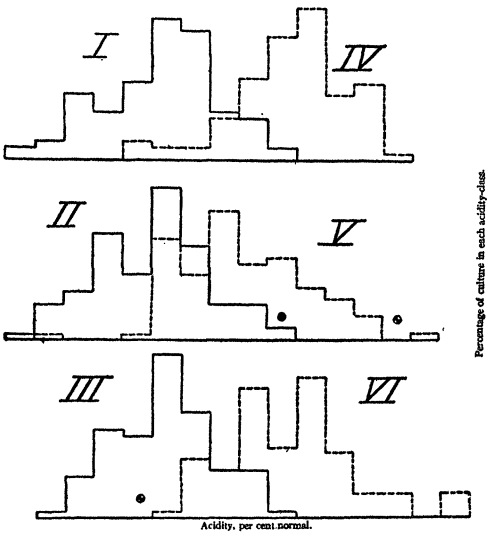

FIG. 1. ACID PRODUCTION BY PARATYPHOID BACILLI. (I.) Distribution of 100 original cultures, Type *A*. (II.) Distribution of 100 subcultures of descendants of maximum strain, Type *A*. (III.) Distribution of 100 subcultures of descendants of minimum strain, Type *A*. (IV.) Distribution of 100 original cultures, Type *B*. (V.) Distribution of 100 subcultures of descendants of maximum strain, Type *B*. (VI.) Distribution of 100 subcultures of descendants of minimum strain, Type *B*.

ment-producing power of some of the red chromogens and the resistance to mercuric chlorid of the *Aurococcus;* and in their paper the similar results of earlier observers are discussed. Such variations as these strongly suggest the pure lines of Johannsen and may perhaps for convenience be designated as pure line variations.

A distinct type of variation is the mutation, a definite sharp variation separated by a wide gap from the normal character of the type, arising spontaneously in a certain regular proportion of the individuals of a race. Barber (1907) has shown for example that peculiar filamentous chains of cells occur, rarely, but with considerable regularity, in cultures of *B. coli,* and that by isolating these filaments and breeding from them he could get a new race, constantly showing the filamentous arrangement and possessing definite cultural characters and a fermentative power considerably higher than the normal. Similar, though less conclusive results were obtained with *B. typhi,* and in one case an apparently non-spore-forming race was derived from *B. megatherium.* A particularly interesting case is that of the fermenting mutants of the typhoid group first observed by Neisser and Massini and recently thoroughly worked out by Penfold (1912). With a number of different strains of typhoid and paratyphoid bacilli and a number of different sugar media it has been shown that an organism which does not ferment the carbohydrate in question may produce on media containing it colonies which after several days bear curious raised papillæ. Subcultures from these papillæ yield a pure culture of a strain which resembles the parent stock in every respect, except that it actively ferments the specific carbohydrate and forms no papillæ; and this mutant breeds true. On the other hand subcultures from other parts of the parent colony produce strains which, like the original stock, do not ferment en masse, but do possess the property of throwing off fermenting mutants. Clark (1913) has recently shown that some at least of these modifications may have been quantitative only rather than qualitative, but the sharpness of the difference involved seems to warrant their recognition as true mutations.

Either fluctuations or mutations may originate as a result of protoplasmic in-

equalities in cell division and without any corresponding variations in environmental condition. Or, on the other hand, they may be causally related to changes in the chemical and physical surroundings of the organism as were those which MacDougal produced among the higher plants by injecting chemicals into the ovary and such as Tower caused by exposing potato beetles to special conditions of temperature and humidity. Changes of this sort are very familiar among the bacteria, as for example in the case of the increase in virulence on passage through susceptible animals, or the converse process of attenuation, as practised in the preparation of vaccines for anthrax and other diseases. Wolf (1909) reports a considerable number of temporary modifications and some permanently inheritable ones stimulated by exposing bacteria to the action of chemicals. White and dark red strains were thus produced from a normal *B. prodigiosus,* the resulting modifications breeding in each case true to their new type. Variations of this sort called forth by the direct effect of the environment I have been accustomed to distinguish by the term "impressed variations."

The net result of the various sorts of variability to which the bacteria are subject is to produce a condition, not different in kind, but more extreme in degree, than that which exists among more complex forms. As Bateson (1913) says: "The problem of species in its main features is presented by these organisms in a form identical with that which we know so well among the higher animals and plants." Several peculiar conditions tend, however, to make specific distinctions even more unstable among the bacteria than elsewhere. In the first place the action of the environment upon unicellular organisms is peculiarly direct and the fact that all cells are potentially reproductive removes any bar against the inheritance of acquired characters. Again the absence of sexual reproduction must operate to preserve variations which arise from within or without. Among sexual organisms it is true that amphimixis is held to be in itself an important source of germinal variations. Yet this is true only within certain rather definite limits and beyond those limits sexual reproduction ceases or becomes infertile and thus the more divergent variations are eliminated. With fission as the normal method of reproduction, on the other hand, every variation which can arise may be handed on, unchanged. Finally the rapidity with which the bacteria multiply furnishes exceptional opportunities for the operation of selection or any other modifying force. The immense number of generations which may succeed each other in a short space of time might be expected to make boundary lines as shifting as they would become among the higher plants if a dozen geological epochs were considered all at once.

There are sharp limits to the variability of even the bacteria however and for practical purposes we find the larger groups quite constant in their general properties. As a rule typhoid germs descend from typhoid germs and tubercle bacilli from tubercle bacilli. The same yellow coccus falls on gelatin plates exposed to the air, all over the world. The same spore-forming aerobes occur in every soil, the same colon bacilli crowd the intestines of animals and men in every clime. In part at least I am inclined to believe that this is due to the direct or selective effect of similar environmental conditions producing what Jordan and Kellogg call among higher organisms "Ontogenetic species held for a number of generations true to a type simply because the environment, the extrinsic factors in the development of all the individuals in these successive generations, are

the same.'' The comparative fixity of the more strictly pathogenic bacteria is a striking illustration of this tendency.

In many instances we find that individual strains of bacteria exhibit an extraordinary uniformity in minor characteristics even when (or perhaps particularly when) cultivated for long periods on artificial media in the laboratory. The two paratyphoid strains, *A* and *B*, described above offer a striking instance of this. The mean acid production of the two strains was respectively 1.4 and 1.6 per cent. normal, differing only by .2 per cent. normal and the fluctuating variations, extending over a range of over 1.0 per cent. in each case, far exceed the mean difference between the strains. Yet subcultures show each strain, as a strain, breeding true to its characteristic. We find slight differences in resistance to unfavorable physical condition or to the action of some chemical disinfectant transmitted unchanged in a particular strain for generation after generation.

As a matter of fact indeed it is not alterations in the characters of bacteria while we are studying them which generally trouble us, but the fact that as we isolate these organisms in nature we find that antecedent variations have produced a bewildering confusion of slightly differing varieties or races or strains. Between well-marked types like *B. coli* and *B. alcaligenes* is a series of forms, each one differing but slightly from its neighbor, but together almost completely bridging the gap between the two extremes. The more refined our methods of bio-chemical examination, the more the types are multiplied, and the more hopeless is the confusion. When Gordon (1905) applied his nine tests to 300 different strains of streptococci, he found 48 different combinations of reactions, and MacConkey (1909) records 36 different varieties of colon bacilli characterized by particular combinations of his seven tests To call each distinguishable strain having definite bio-chemical properties a species and to give it a name of its own, is quite out of the question. To ignore all minor differences and maintain as specific such complex groups as are included under the term *B. coli* or *Str. pyogenes* is misleading and an effective bar to future progress.

The first principle, which has proved of prime assistance in the characterization of bacterial types, and which offers a rational compromise between either false unity or bewildering multiplicity, is the recognition of the fact that types which occur commonly among bacteria as they are found in nature are of greater systematic importance than those which occur rarely and occasionally. Of course from one standpoint every inheritable protoplasmic variant which exists is of equal importance with every other. For practical purposes, however, we must recognize certain types as "species" or "varieties" even though they may sometimes intergrade. Among the higher plants and animals such systematic units are usually recognized on the basis of discontinuity in some definite character. The more refined methods of biometry have however revealed another grade of kinds (I use this word to avoid the artificial implications of "species" or "variety"), marked by relative rather than absolute discontinuity. Frequently the measurement of some particular differential character and the plotting of a "frequency polygon," with grades of the chosen character as abscissæ and the proportion of individuals showing each grade as ordinates, shows a curve with two distinct peaks, a bimodal curve. The studies reported by Bateson on the length of the cephalic horns of the rhinoceros beetle and on the forceps length of the earwig and De Vries's observations on the petals of a chrysanthemum, are excellent examples.

In each case the peaks on the curve indicate distinct centers of variation around which the intermediate fluctuations are grouped and these constitute biologic facts of real importance, even though the types overlap and appear to blend in the valleys between the modes.

Johannsen in his recent book (1913) has discussed such bimodal curves with admirable clearness and points out that obvious phenotypes (externally recognizable kinds) may or may not represent true genotypes (characterized by germinal differences),—

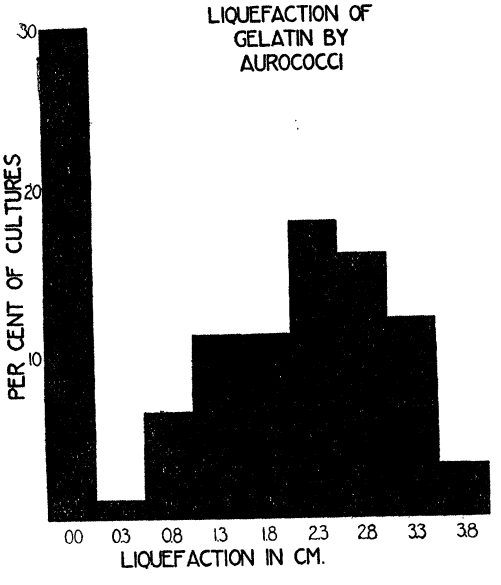

Fig. 2.

breeding experiments being the only final criterion. In our bacteriological work we are making breeding experiments all the time and even though our study of mass reactions may be crude, it is free from the grosser errors due to environmental variations, and our bimodal curves indicate real protoplasmic diversity. For example, in a study of the gelatine liquefying power of the orange cocci, it appeared that the depth of liquefaction after 30 days varied from 0 to over 3.5 cm., each intermediate .5 c.c. value being represented. Yet the frequency with which various grades of liquefaction occurred showed that only two distinct types were common (Fig. 2), one failing entirely to liquefy, including 30 per cent. of the strains studied, and the other liquefying rapidly, to the extent of 2.0–3.5 cm., including 45 per cent. of the strains. Ordinarily such a difference in proteolytic power as that between a liquefaction of 1.0 cm. and one of 3.0 would be considered important as marking a distinction between a very slowly and a rapidly liquefying type. Yet in view of the frequency curve it is both practically convenient and biologically sound to say that we are dealing with two and only two distinct types, so far as this character is concerned, one not liquefying at all and the other liquefying vigorously to an extent of 2.0–3.5 cm. in 30 days, while slowly liquefying strains may be considered as aberrant varieties.

Another example of this conception of frequency types may be taken from recent studies of the fermentative power of the colon bacilli and the streptococci. Both these groups have been split up according to their acid-producing power in a wide variety of carbohydrate media and any one sugar has been considered just as important as any other, giving almost as many types as there are permutations and combinations of the test substances used. Howe (1912) has shown for the colon bacilli, and the same thing is true for the streptococci (Winslow, 1912), that the various carbohydrate media are not fermented at random, but stand to each other in a definite "order of availability" forming what Howe calls a "metabolic gradient," such that if any member of the series is fermented the chances are that those ahead of it will be fermented also. Thus in the colon group dextrose is most often attacked, then lactose, then saccharose and then raffinose. Certain steps in the gradient are qualita-

tively far more important than others. Of 540 dextrose fermenting bacilli freshly isolated from the intestine Howe found that practically all attacked lactose. Saccharose divided the group into two approximately equal subgroups, 58 per cent. attacking this sugar and 41 per cent. failing to do so. Of the 58 per cent. all but 5 per cent. also attacked raffinose so that the dextrose-lactose-saccharose forms may be considered intermediate variants between the two main divisions which ferment either dextrose and lactose alone or all four sugars (Fig. 3). It seems clear that such quantitative studies furnish the surest basis for deciding which sugars are primarily important in classification and we may safely conclude that so far as these four sugars are concerned there are only two important divisions of the colon group. The use of dulcite which occupies an equally prominent place in the classification of MacConkey (1909) and Jackson (1911), rests on no such biometrical basis and we have no good reason to think it deserves special importance. In the same way the admirable study by Stowell, Hilliard and Schlesinger (1915) of the streptococci shows that the five groups fermenting respectively dextrose only, dextrose and lactose, dextrose, lactose and saccharose, dextrose, lactose, saccharose, and raffinose and dextrose, lactose, saccharose, raffinose and salicin are quantitatively of special importance and include between them 68 per cent. of 240 strains studied.

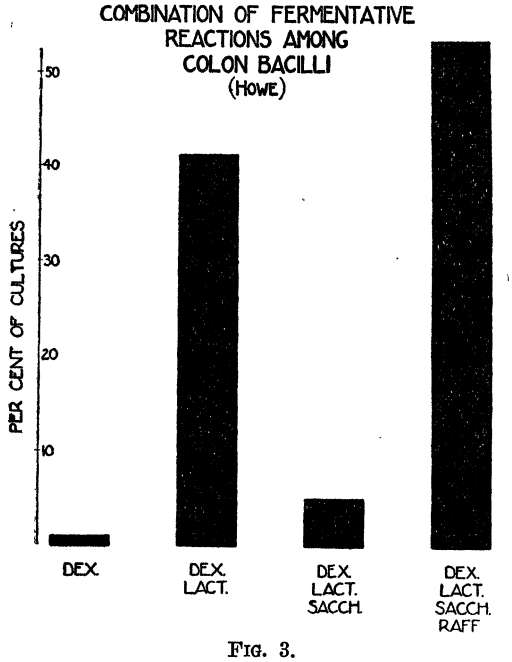

FIG. 3.

A second conception, of much assistance in the classification of bacteria, is the principle that special weight should be given to characters quantitative or qualitative, which are found to be correlated with each other in a number of different types. The principle of numerical frequency offers a basis for characterizing the individual types and the principle of correlation a basis for classifying these types in accord with their biological relationships.

The early bacteriologists established a dozen genera, such as *Streptococcus, Sarcina, Bacillus, Bacterium,* and the like, based entirely on a few obvious morphological characters. Some of these genera are undoubtedly valid. Others like those which are based only on the presence or absence of flagella are quite as certainly invalid. No one familiar with the colon group can hold that it is reasonable to place the common type of motile colon bacillus in the genus *Bacillus* along with *B. mycoides, B. aerogenes, B. anthracis, B. prodigiosus, B. radicicola* and *B. tetani* and to place an organism having all its other properties identical but lacking flagella in the genus *Bacterium.* The same arguments hold true against the genera *Planococcus* and *Planosarcina* among the cocci. We find in several of the major groups motile and non-motile forms which are precisely alike in half a dozen respects and are clearly minor varieties of the same

type, and it is absurd to give them generic rank, and group together widely different types which are alike in no single respect except that they have flagella. These genera based on motility are on a par with a division of animals into those with wings and those without, which would place bats and birds and flying fishes and bees in one group and cats and ordinary fishes and worker ants in another.

The unsatisfactory nature of the Migula classification, which, even if the motility genera were accepted, left over one third of all known bacteria in the genus *Bacillus*, led many bacteriologists to abandon any attempt at a natural classification and to seek refuge in frankly arbitrary schematic groupings. The logical outcome of this tendency is the decimal group number which our society has adopted, the history of which has been so ably presented by Professor Harding (1910).

The group number, according to which the characters of bacteria are indicated by a conventional series of decimals, has an undoubted value and has proved a godsend to workers who study a large series of new cultures and desire a concise record of their behavior. It is a sort of index to the chief characters of the organisms in question, a method of cataloging reactions observed. It is obvious however that it is artificial, and that it does not furnish a "classification," an arrangement of bacteria according to their natural relationships.

There is some danger, I think, that this important distinction between the group number on our standard card and a real biological classification may be forgotten. When the student notes that 100 means that endospores are produced and 200 that they are not produced, he is likely to draw the conscious or subconscious conclusion that all bacteria producing endospores are more closely related to each other, are more of a kind, than are the members of the two separate groups. I think that this is very probably a fact. Then, of the non-spore formers, he notes that strict aerobes fall under 210, strict anaerobes under 230 and facultative forms under 220. Again he is likely to draw a similar conclusion as to relative relationships and again perhaps the conclusion is reasonably correct. In the third place of the whole numbers, however, any such deduction as to natural relationships from the group number would certainly be erroneous. Of the facultative non-spore formers, all which liquefy gelatine fall under 221; and all which fail to do so under 222. That is, the group number throws together on the one hand *B. cloacæ* and the liquefying strains of fluorescent water bacteria and the liquefying proteus forms, and on the other hand *B. coli* and the non-liquefying fluorescent and proteus types. It is reasonably certain however that liquefying and non-liquefying fluorescent bacteria are more closely related to each other, that *B. coli* and *B. cloacæ* are more closely related to each other, that liquefying and non-liquefying proteus types are more closely related to each other than are the liquefying or the non-liquefying members of the three respective pairs. Precisely as in the case of Migula's motility genera the use of a single arbitrarily chosen character in classification leads to misleading results.

It is sometimes held that the difficulties we experience in bacterial classification are due to the fact that we must necessarily rely in the main on physiological rather than on morphological characteristics. I do not believe this to be the case. There is no fundamental distinction between morphological and physiological properties, since all are at bottom due to chemical differences in germ plasm, whether they happen to manifest themselves in the size and arrangement of parts or in the ability

to utilize a certain food stuff. Indeed biochemical properties have a peculiar and unique significance among the bacteria, since it is precisely along the lines of metabolism that these organisms have attained their most remarkable differentiation. The higher plants and animals have developed complex structural modifications to enable them to obtain food materials of certain limited kinds. On the other hand the bacteria have maintained themselves by acquiring the power of assimilating simple and abundant foods of varied sorts. Evolution has developed gross structure in one case without altering metabolism; it has produced a diverse metabolism in the other case, without altering gross structure. There is as wide a difference in metabolism between the pneumococci and the nitrifying bacteria as there is in structure between a liverwort and an oak. The danger in using physiological characters for classification lies, not in their inherent unreliability, but in the fact that so many physiological properties are directly adaptive in nature. Adaptive characters of similar nature are likely to arise in different groups under the influence of similar environmental conditions and may prove altogether misleading as to true phylogenetic relationships. Professor Gadow in his striking address before the British Association has called the independent evolution of a nearly identical character from homologous material isotely. We have excellent examples among the bacteria. It seems clear for example that we must assume from the presence of liquefying and non-liquefying types among so many of the principal groups of bacteria that this property has lain latent in a great many independent lines of descent and has been independently released in many of them, perhaps by environmental forces. It is particularly to avoid this danger of confusing independently acquired adaptive characters with those which indicate real community of descent that we must lay stress on the significance of a number of independent characters which occur in correlation. If the correlation is due to an essential dependence of one character upon the other it is of course not particularly significant; but, when we find a number of different characters, which have no necessary connection, correlated together, the presumption is warranted that common descent is the connecting link which has united them.

It was in the study of the Coccaceæ that the full importance of emphasis on correlated characters first impressed itself upon me. It had long been the practise, following the Migula system, to group all the staphylococci of the skin and the saprophytic cocci which divide in one and two planes together in the genus *Micrococcus* and to separate the packet-formers in the genus *Sarcina*. The common cocci found on the skin, all liable to assume at times pathogenic properties, were usually classed as merely three color varieties (*aureus, albus* and *citreus*) of a single species *Micrococcus* or *Staphylococcus pyogenes*. In the attempt to apply statistical principles to the classification of this group,[1] Mrs. Winslow and I collected and studied 500 different strains of cocci, measuring quantitatively so far as possible eleven different characters of each strain. At once a new and surprising set of relationships manifested themselves. It was evident in the first place that on the whole the cocci living normally on the body surfaces, differed in almost every respect from the cocci

[1] First published in Biological Studies by the Pupils of William Thompson Sedgwick, June, 1906, and in the *Journal of Infectious Diseases* for the same year and later elaborated in our book on the "Systematic Relationships of the Coccaceæ," N. Y., 1908.

of the water and earth. The former usually occurred in chains or small irregular groups, reacted positively to the Gram strain, formed a meager or only fair surface growth on solid media, and produced considerable acid in carbohydrates. The cocci of the water and earth occurred in large cell groups or packets, never in chains, were usually Gram negative, grew abundantly on solid media and generally failed to ferment carbohydrates. There were exceptions of course, as there always must be in an unstable group like the bacteria. Some organisms which a general consideration of all their characters would place in the latter group were found on the skin, while others were Gram positive or fermented the sugars. Yet on the whole the relation seemed a sufficiently definite one to warrant the division of the spherical bacteria into two subfamilies, the Paracoccaceæ and the Metacoccaceæ. The next thing which was apparent was that the color of the pigment produced, instead of being a minor varietal character, was fundamentally correlated with other properties which were apparently of sufficient importance to deserve generic rank. It appeared that the orange and white staphylococci, along with the diplococci and streptococci, all shared the properties of the Paracoccaceæ just enumerated, while the yellow and red pigment formers (in spite of the occasional presence of the former on the skin and even in connection with pathological processes) exhibited the characters of the Metacoccaceæ (Fig. 4). The white and orange forms further differed from each other in the fainter surface growth of the former and in the important fact that liquefying members of the orange series liquefy twice as rapidly as do the liquefying white strains. Hence we distinguished these groups as the genera *Aurococcus* and *Albococcus*. Among the Metacoccaceæ the yellow and red forms were sharply separated by the much higher proportion of strains which reduce nitrates to nitrites and by the absence of ammonia formation in nitrate pepton broth and by the rarity and slowness of liquefaction, among the red chromogens, for which we

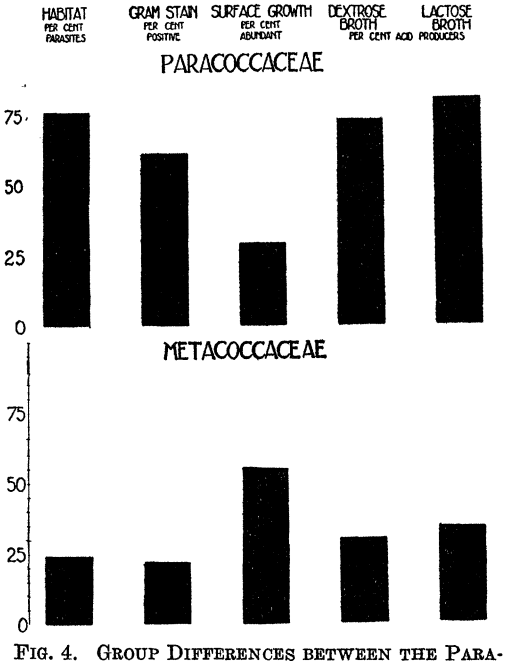

FIG. 4. GROUP DIFFERENCES BETWEEN THE PARACOCCACEÆ AND THE METACOCCACEÆ. The upper blocks show for 221 strains of cocci whose type of chromogenesis (white or orange) would place them with the parasitic subfamily, the per cent. of strains fulfilling each of five individual criteria of that group. The lower blocks show for 279 strains of cocci whose type of chromogenesis (yellow or red) would place them with the saprophytic subfamily the per cent. of strains fulfilling each of five individual criteria of that group.

suggested the generic name *Rhodococcus*. The important point brought out by these studies was that chromogenesis and the Gram strain, characters which we all had believed to be comparatively unimportant, proved to be correlated with a number of other properties and therefore highly significant. On the other hand the Migula

distinction between *Micrococcus* and *Sarcina* appeared to be of quite minor importance. Among the yellow chromogens we found a completely parallel series of packet-formers and non-packet formers exactly alike in all other respects. So with liquefaction of gelatine. Among aurococci, albococci, micrococci and sarcinæ were strains having all other properties in common, but differing in this one respect. Kligler (1913) has recently examined the cocci in the American Museum collection and has found that the fifty strains represented fall very clearly in the genera thus outlined, although he concludes that the species originally described should be modified in certain important respects.

The general principles of statistical classification were outlined as follows by Miss Rogers and myself (1906):

> We have first plotted the frequency curve for each character in order to find whether the array varies about one or several modes, and where these modes are situated. . . . In the second place, we have calculated correlation factors for the most significant pairs of characters. Each mode on the curves of frequency may fairly be taken to mark a natural species or variety, and the characters which vary together must form the most important basis for the establishment of the larger groups. By such a method alone it is possible to locate those mountain peaks in the chain of bacterial variations which rightly deserve generic and specific names.

In the same year in which this paper was published, Andrewes and Horder in England presented a revision of the species in the genus *Streptococcus* (1906) based on exactly identical principles at which they had independently arrived. They say:

> There was, however, one guide which, as in all such taxonomic problems, proved of the greatest help, namely, the numerical frequency of occurrence of any given type. . . . The common types stand out as mountain tops above their fellows, each mountain connected by valleys of intermediate types with many of its neighbors.

Since these suggestions were first made, the statistical method has been systematically applied by Howe (1912) to the colon group, by Stowell, Hilliard and Schlesinger (1913) to the streptococci, by Dr. Morse (1912) to the diphtheria group and by Rogers and Davis (1912) to the lactic acid groups.

There are many other serious investigations of bacterial relationships which might be cited, many of them made before the term "statistical classification" was thought of in this connection, but characterized by the fact that they include a careful comparative study of many different strains with due regard to the frequency with which types occur and to the special importance of correlated characters. Among the earlier investigations, Beijerinck's study of the acetic acid bacteria (1898), Chester's on the aerobic spore formers (1904) and Hefferan's on the red pigment producers (1904) are worthy of special mention. More recently Edson and Carpenter have given us an excellent revision of the group of fluorescent bacteria (1912). Owen (1911) has added much to our knowledge of the aerobic spore formers, White (1909) has revised the *B. bulgaricus* group and Dr. Claypole (1913) has worked out some very striking correlations between immunity relations and cultural characters among the streptothrices. The elaborate study of the dysentery group by the late Dr. Hiss (1904) and Elser and Huntoon's review of the Gram-negative cocci (1909) should also be mentioned in this connection. There has already been accumulated a considerable mass of data which when critically examined and codified should furnish a good basis for a systematic arrangement of many of the smaller bacterial groups.

So far as the general classification of the bacteria into larger groups, families and

genera, is concerned, we have also, I believe, plenty of information which if properly digested would make it possible to arrive at reasonable and helpful results. With these large groups no special statistical study is generally necessary, for the chief characters of the major types are well established. All that is needed is interpretation, but interpretation based on a view of all the available facts and on a sound conception of biological principles. Until recently the only attempt at a general classification of the bacteria based on a common-sense interpretation of all the characteristics of the organisms is that presented by Flügge (1896), the chief value of which lay in the classification of the rod-shaped bacteria into 22 groups, almost all of which appear to represent natural aggregations of allied types. For example, we all recognize that the aerobic spore formers, the anaerobic spore formers, the colon-typhoid group, the nitrifying organisms, the fluorescent bacteria, and the group of diphtheria and tubercle bacilli constitute real groups of related organisms.

Four years ago a more ambitious attempt at a fundamental analysis of the systematic relationships of the whole group of bacteria was made by Professor Orla Jensen, of the Polytechnicum of Copenhagen (1909). Professor Jensen with good reason discards the purely morphological basis of classification and in particular the distinction based on the presence or absence of flagella. The arrangement of flagella when they are present, on the other hand, offers a convenient index of other more important differences and Professor Jensen gives his two orders of bacteria the names of Cephalotrichinæ (monotrichous or lophotrichous) and Peritrichinæ (peritrichous). The Cephalotrichinæ, deriving their life energy almost entirely from oxidative processes, are all water or moist earth forms, with the exception of a few peculiar plant and animal parasites and for the most part grow badly or not at all on ordinary organic media, and spores are never formed. The series begins with the Oxydobacteriaceæ, including the most primitive bacteria, which oxidize methane and carbon monoxid, the nitrifiers, the acetic acid bacteria and the Azotobacter group. Then follows the Actinomyces family which includes the root nodule bacteria and the mycobacterium (tuberculosis) group. The collocation of the latter forms is startling at first, but their morphology, their oxygen requirements and their unique pathological relations, almost symbiotic by contrast with the quick toxic action of other pathogenic bacteria, offer some evidence of real relationship. The third, fourth and fifth families are the Thiobacteriaceæ (the sulfur bacteria), the Rhodobacteriaceæ (the red or purple sulfur bacteria) and the Trichobacteriaceæ (*Cladothrix, Crenothrix, Beggiatoa,* etc.) which are clearly natural groups. The last two families, the Luminibacteriaceæ and the Reducibacteriaceæ, are typically denitrifying organisms which form a connecting link between the primitive oxidizing bacteria and the Peritrichinæ. They include the fluorescent water bacteria and the phosphorescent vibrios and at the higher end of the series such forms as the cholera organism in which the ability to split complex products with the formation of lactic acid and indol begins to appear.

The second order, the Peritrichinæ, includes the more specialized bacteria in whose metabolism the splitting of carbohydrates or amino-acids plays a primary rôle rather than oxidation or denitrification. They are rods or cocci, peritrichous when possessing flagella at all, and among them are found all the commoner putrefactive and parasitic types. This order, according

to Jensen, may be divided into four families. The first, the Acidobacteriaceæ, includes the non-spore-forming carbohydrate fermenting types, among the principal representatives being the colon-typhoid group and practically all the cocci. His second family, the Alkalibacteriaceæ, shows a higher development of the power of decomposing nitrogenous bodies, and includes the liquefying proteus forms, the actively liquefying aerobic spore formers and certain urea fermenters. The last two families, the Butyribacteriaceæ and the Putribacteriaceæ, are made up of the strict anaerobes.

Whatever minor criticisms may be made of Professor Jensen's scheme, I believe that no one who has thought seriously about bacterial relationships can study it carefully without feeling that it is by far the most successful attempt yet made at a real biological classification of the group and that future progress will probably consist in its modification and extension rather than in any profound reversal of its basic principles.

Inertia in terminology is strong and it is the business of no one in particular to criticize and report on the value of suggestions as to bacterial classification and nomenclature. We are all too busy with our own special field to undertake such a task of our own accord. Yet I believe that in the present state of bacteriology such a critical examination of suggested systematic arrangements is most essential. A mass of work has been done in the last ten years, potentially valuable, but almost useless so long as it remains a sealed book to all but its respective authors and their pupils.

What we need at this time is a court of appeal on matters of systematic bacteriology, a court to which all suggested classifications, past and future, may be referred for official acceptance or rejection, in whole or in part. Such a court or commission might take first Professor Jensen's classification as the most recent comprehensive attempt to treat the whole group of the bacteria, and after careful consideration might adopt such of his families and genera as seem well established and issue a report in which they should be definitely and clearly defined. Such a report from a commission of a proper caliber would not be ignored as work of any single worker may be, but would be adopted and would become at once a part of the practical working machinery of our science. The genera and species suggested for the Coccaceæ, the Andrewes and Horder species of the genus *Streptococcus,* Chester's species of spore-bearing aerobes, Dr. Morse's types of pseudo-diphtheria bacilli, Edson's types of fluorescent bacteria, etc., might be later taken up so that ultimately a complete scheme of bacterial classification would be at our disposal.

Such authoritative commissions on classification and nomenclature are well established in the older biological sciences, as for example, the International Commission on Zoological Nomenclature appointed by the Third International Zoological Congress in 1895 and made permanent at the Fourth Congress in 1898. Its work has been much more along the line of precise legal definitions and the determination of priority in terminology, than would be the case with a similar commission in bacteriology. The broader constructive work which has already been accomplished in zoology still remains for us to do. Furthermore we have no international congress to which such a commission could profitably report on all the phases of its work, although for one group of the bacteria, the colon-typhoid group, a commission on systematic relations was created by the last International

Congress on Hygiene and Demography, of which Dr. Weber, of Berlin, is chairman and the president and two past presidents of this society are the American members. I believe this to be a fitting time for a more far-reaching attempt to criticize and collate and systematize the work which has been done in many countries and by many observers on the characterization and classification of bacterial types. The inception of such a plan may very properly come from the Society of American Bacteriologists which through its standard card has already done so much for the development of the purely descriptive side of our science. As a practical outcome of this long survey of the problems of systematic bacteriology, with which you have borne so patiently, I suggest that we invite fifteen bacteriologists from the principal scientific countries to act as an international commission on the characterization and classification of bacterial types, with the general objects outlined above. If you should approve this plan, and if we can secure the cooperation of investigators of the first rank (as I have no doubt will be the case), I believe that we can thus render to bacteriology a great practical service, worthy of the highest aims which our society has held in view.

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ACADEMIC STUDENT ELECTIONS

IN SCIENCE, October 24, 1913, are some interesting tables exhibiting the results of much patient work by Dr. Frederick C. Ferry, dean and professor of mathematics in Williams College. His tables give the registration of students taking various subjects of study in eighteen American colleges and universities. These subjects are commonly divided into three groups, roughly determined by the nature of the topics included. Thus, group I. may be called the "language group"; group II., the "humanities group"; and group III., the "science group." The distribution of work in each of these groups in any given college affords some indication of the popularity of the group. To express this distribution it is necessary to give, as Dean Ferry has done, for each subject the number of "student hours of instruction." In view of the great variation in total attendance at different colleges Dean Ferry has reduced his figures to percentages, on a semester basis, limiting them to undergraduates in the academic college.

On examining Dean Ferry's tables the present writer perceived the possibility of making the comparisons somewhat more pointed, and of securing a fair representation of popularity not only for groups but for separate subjects of study. A "student-hour of instruction" may be interpreted to mean one hour per week in the classroom, taken by one student throughout one semester. The actual work done includes an estimated pair of hours spent in study in preparation for the work of the classroom. This estimate is often not realized, the student taking his chances of escaping a test, especially if the class is rather large. Two or three hours in the laboratory are hence fairly counted as the equivalent of one hour in the classroom. Let h denote the value in student-hours for a given course; for example, if the student has 3 meetings per week in the classroom and 2 afternoons per week in the laboratory, then $h = 5$. Let n denote the number of students taking this course during a given semester; then nh denotes the work done in this course.

Now, let $\Sigma(nh)$ denote the work done in the sum of all the courses of a given subject; for example, there may be four courses in physics. Let A denote the whole academic work in stu-