Technical and Bibliographic Notes/Notes techniques et bibliographiques

The Institute has attempted to obtain the best original copy available for filming. Features of this copy which may be bibliographically unique, which may alter any of the images in the reproduction, or which may significantly change the usual method of filming, are checked below.

L'Institut a microfilmé le meilleur exemplaire qu'il lui a été possible de se procurer. Les détails de cet exemplaire qui sont peut-être uniques du point de vue bibliographique, qui peuvent modifier une image reproduite, ou qui peuvent exiger une modification dans la méthode normale de filmage sont indiqués ci-dessous.

- [x] Coloured covers/ Couverture de couleur
- [ ] Covers damaged/ Couverture endommagée
- [ ] Covers restored and/or laminated/ Couverture restaurée et/ou pelliculée
- [ ] Cover title missing/ Le titre de couverture manque
- [ ] Coloured maps/ Cartes géographiques en couleur
- [ ] Coloured ink (i.e. other than blue or black)/ Encre de couleur (i.e. autre que bleue ou noire)
- [ ] Coloured plates and/or illustrations/ Planches et/ou illustrations en couleur
- [ ] Bound with other material/ Relié avec d'autres documents
- [ ] Tight binding may cause shadows or distortion along interior margin/ La reliure serrée peut causer de l'ombre ou de la distortion le long de la marge intérieure
- [ ] Blank leaves added during restoration may appear within the text. Whenever possible, these have been omitted from filming/ Il se peut que certaines pages blanches ajoutées lors d'une restauration apparaissent dans le texte, mais, lorsque cela était possible, ces pages n'ont pas été filmées.
- [ ] Additional comments:/ Commentaires supplémentaires:

- [ ] Coloured pages/ Pages de couleur
- [ ] Pages damaged/ Pages endommagées
- [ ] Pages restored and/or laminated/ Pages restaurées et/ou pelliculées
- [x] Pages discoloured, stained or foxed/ Pages décolorées, tachetées ou piquées
- [x] Pages detached/ Pages détachées
- [x] Showthrough/ Transparence
- [ ] Quality of print varies/ Qualité inégale de l'impression
- [ ] Includes supplementary material/ Comprend du matériel supplémentaire
- [ ] Only edition available/ Seule édition disponible
- [ ] Pages wholly or partially obscured by errata slips, tissues, etc., have been refilmed to ensure the best possible image/ Les pages totalement ou partiellement obscurcies par un feuillet d'errata, une pelure, etc., ont été filmées à nouveau de façon à obtenir la meilleure image possible.

This item is filmed at the reduction ratio checked below/
Ce document est filmé au taux de réduction indiqué ci-dessous.

10X	12X	14X	16X	18X	20X	22X	24X	26X	28X	30X	32X
						✓					

The copy filmed here has been reproduced thanks to the generosity of:

Medical Library
McGill University
Montreal

The images appearing here are the best quality possible considering the condition and legibility of the original copy and in keeping with the filming contract specifications.

Original copies in printed paper covers are filmed beginning with the front cover and ending on the last page with a printed or illustrated impression, or the back cover when appropriate. All other original copies are filmed beginning on the first page with a printed or illustrated impression, and ending on the last page with a printed or illustrated impression.

The last recorded frame on each microfiche shall contain the symbol —► (meaning "CONTINUED"), or the symbol ▼ (meaning "END"), whichever applies.

Maps, plates, charts, etc., may be filmed at different reduction ratios. Those too large to be entirely included in one exposure are filmed beginning in the upper left hand corner, left to right and top to bottom, as many frames as required. The following diagrams illustrate the method:

L'exemplaire filmé fut reproduit grâce à la générosité de:

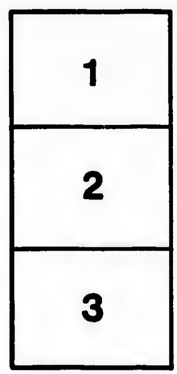
Medical Library
McGill University
Montreal

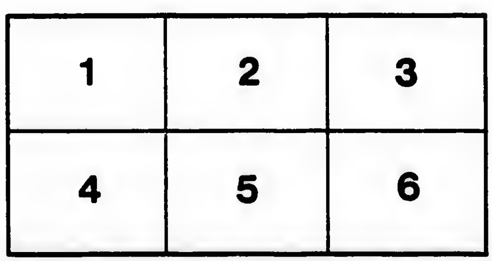
Les images suivantes ont été reproduites avec le plus grand soin, compte tenu de la condition et de la netteté de l'exemplaire filmé, et en conformité avec les conditions du contrat de filmage.

Les exemplaires originaux dont la couverture en papier est imprimée sont filmés en commençant par le premier plat et en terminant soit par la dernière page qui comporte une empreinte d'impression ou d'illustration, soit par le second plat, selon le cas. Tous les autres exemplaires originaux sont filmés en commençant par la première page qui comporte une empreinte d'impression ou d'illustration et en terminant par la dernière page qui comporte une telle empreinte.

Un des symboles suivants apparaîtra sur la dernière image de chaque microfiche, selon le cas: le symbole —► signifie "A SUIVRE", le symbole ▼ signifie "FIN".

Les cartes, planches, tableaux, etc., peuvent être filmés à des taux de réduction différents. Lorsque le document est trop grand pour être reproduit en un seul cliché, il est filmé à partir de l'angle supérieur gauche, de gauche à droite, et de haut en bas, en prenant le nombre d'images nécessaire. Les diagrammes suivants illustrent la méthode.





ON THE VARIABILITY OF BACTERIA

AND THE

DEVELOPMENT OF RACES.

BY

J. G. ADAMI, M.A., M.D.,

Fellow of Jesus College, Cambridge.

[*Reprinted from the "Medical Chronicle," September, 1892.*]

JOHN HEYWOOD, PRINTER, MANCHESTER AND LONDON.

ON THE VARIABILITY OF BACTERIA

AND THE

DEVELOPMENT OF RACES.

BY

J. G. ADAMI, M.A., M.D.,

Fellow of Jesus College, Cambridge.

(*Reprinted from the "Medical Chronicle," September, 1892.*)

JOHN HEYWOOD,
DEANSGATE AND RIDGEFIELD, MANCHESTER;
2, AMEN CORNER, LONDON, E.C.

ON THE VARIABILITY OF BACTERIA AND THE DEVELOPMENT OF RACES.*

FROM a medical, as from a biological point of view, the variability of micro-organisms is a subject of the highest interest. If it can be clearly demonstrated that modifications of varying degrees of permanency can be induced in sundry species of bacteria, that new "races" are capable of being developed under the influence of conditions that are easily recognisable and easily controlled, then not only do we gain further knowledge of the laws governing evolution, but—what for us is of more immediate import—we become enabled to clear up not a few of the difficulties constantly presenting themselves in the study of zymotic, or mycotic, disease. The class of difficulties to which I refer is so familiar that here I need give but the briefest indication thereof. It is notorious, for instance, that successive epidemics of one disease, say scarlatina or influenza, are characterised by well marked differences in symptomatology, and in the intensity of effects upon the individual; it is notorious also that during the progress of one epidemic remarkable changes are to be made out in the virulence of the malady. Often at the onset the cases are so mild, the symptoms so vague, that diagnosis can only be pronounced with exceeding caution. Such doubtful early cases may be followed by a succession in which the disease is typical and of increasing virulence, and eventually is reached the period of decline of the epidemic, and now again the cases assume a dubious aspect with indefinite atypical symptoms. Or take a disease which now unfortunately has become more or less endemic, namely, diphtheria, and observe the series of progressive cases that is to be made out from the simple sore throat associated with the mildest constitutional effects, through slight cases of croup to those terribly malignant attacks in which the manifestation of the disease and death are almost synchronous.

If the micro-organisms of disease have constant attributes, if the species of bacteria are fixed, their characters unvarying, then all these

* Thesis for the degree of M.D. Cambridge, portions of which, in a modified form, accompanied by bacteriological demonstrations, were contributed to the Pathological Section of the British Medical Association at Nottingham, July, 1892.

differences in the nature and intensity of the symptoms produced by any one micro-organism at diverse periods and in diverse individuals can only be due to subtle meteorological changes, to differences in the power of resistance to microbic invasions displayed by the individual and to the dose, if I may so term it, of infective material incepted by the individual. Now, without doubt, all these factors are of *very great importance*, but at the same time it cannot be denied that they do but vaguely advance our knowledge. Up to the present the study of atmospheric influences as affecting zymotic diseases, while impressing upon us one or two main truths, has, in other respects, involved us in a maze of contradictions, and while the last few years have greatly extended our knowledge of the nature of the conflict between the organism and the microbe, clinical experience is constantly bringing before us the paradoxical phenomenon of disease in its severest form attacking individuals in whom one would hold the constitutional powers of resistance to be at their highest. So, too, difficulties in the way of exact determination are insuperable, clinically, with reference to the "dosage" of infective material.

But if, while granting freely that all the above-mentioned factors play each a part, it can be shown that modifications of the powers of micro-organisms can be induced, both within the organism and outside it—alterations in appearance, in the ferments and other products of their vital activity and (among pathogenic microbes) in virulence; alterations, not temporary, but continuing through generations; alterations that are capable of being demonstrated in the test-tube and under the microscope—then we reach one stage further in actual advance in our knowledge of infectious disease, and from this advance gain a firmer standpoint from which to appreciate the other factors.

Largely influenced by the admirable researches of the German bacteriologists, and in this country by the teaching of Dr. Klein, the tendency of the medical world has been to regard the species of micro-organisms, and especially of pathogenic micro-organisms, as fixed. If in each case of a given disease a given microbe is discovered with specific cultural characteristics, if the more closely zymotic disease is studied the more clear becomes the evidence of its infectious nature, of its passage either directly or indirectly from person to person, while the more the subject is studied the weaker becomes the evidence of the spontaneous origin of infectious disease, then the greater is the difficulty of arriving at any other conclusion than that pathogenic microbes belong to species that are fixed and possess unchanging attributes. I need not say how valuable, not to say necessary, it has been in a young science like bacteriology to hold this view of the fixity of species, and, speaking broadly, this view must still be held. In the great majority of cases the micro-organisms gained from the tissues of the patient suffering from any one zymotic disease approximate sufficiently closely to the type for us to say with absolute confidence that no specific alteration is to be discovered in them. But while acknowledging this much it must be confessed that there has been a tendency to overlook differences in the mode of growth of pathogenic microbes—generally, it is true, minute, though not always so—and to dwell upon differences in the resistance of the organism more than upon differences in the pathogenic properties of the micro-organisms whenever, in an individual case, or in an epidemic, the symptoms have varied from the ordinary. While bacteriologists in general admit, to a greater or less extent, that bacteria show variations, I know of no work in which the facts in connection with such variations have been brought together; it may, therefore, be useful at the present time if I gather together some of the many researches into the variability of microbes, and show that this variability can clearly be made out.

Undoubtedly where a number of facts are brought together, all pointing in one direction, there is a danger that by some these facts will be held to support an extreme theory, and one that cannot surely be gained from them—the theory, in this case, that non-pathogenic bacteria can easily become pathogenic, and that zymotic disease may, as a frequent event, arise *de novo.* While, possibly, further research may prove that in a certain limited number of cases a harmless saprophyte is capable of developing into a pathogenic microbe, at the present moment the facts are wanting to support this hypothesis. The extreme conclusion to be reached now is that there are species of pathogenic micro-organisms whose virulence is capable of manifesting great modification, and that such modification is fitted to explain some of those differences in the course of zymotic disease which must have arrested the attention of all medical men.

ON CERTAIN CHARACTERISTICS OF THE BACTERIA AS A WHOLE AFFECTING THE LIABILITY TO VARIATION.

At the very onset we should expect to find the bacteria peculiarly well-adapted for the study of the most elementary problems of evolution; their organisation, as compared with other forms of life, is so simple, the generations—if generations they may be called*—succeed each other with such extraordinary rapidity, and, what is of yet greater importance, our study of these forms would seem to be wholly freed from the per-

* The word generation as applied to the Schizomycetes is not a little unsatisfactory—yet I know no other word at present in use that is capable of replacing it. Apart from the sexual significance that may attach to it, this word embraces the idea of parentage. Now, among the bacteria there is no true parentage, for what happens in the process of fission is that the bacillus divides into two portions, each of which becomes a separate individual, but neither can be regarded as the parent form; each equally with the other, represents—and, to a certain extent, is—the earlier single individual.

turbing influence of sex. On the one hand there can, in the bacteria, be no tendency towards the production of races through sexual agency; on the other the reverse tendency is equally absent, namely, the tendency of conjugation to preserve the mean, to lead to the development of individuals approximating to the type.

Here, then, in studying these unicellular or sexual bacteria, we investigate the simplest and most fundamental principles of heredity and evolution, and the influence of environment becomes most evident. Weismann, who in the higher forms of life would make the development of individual differences and the inheritance of the same almost wholly dependent upon the phenomena which accompany the fusion of the male and female reproductive cells, freely acknowledges that to the protozoa we must look for the origin of "hereditary individual variability," and, dealing with them, emphasises this influence of environment. "If, for instance, a protozoon, by constantly struggling against the mechanical influence of currents in water, were to gain a somewhat denser and more resistant protoplasm, or were to acquire the power of adhering more strongly than other individuals of its species, the peculiarity in question would be directly continued on into its two descendants, for the latter are at first nothing more than the two halves of the former. It therefore follows that every modification which appears in the course of its life every individual character, however it may have arisen, must necessarily be directly transmitted to the two offspring of a unicellular organism."*

And in connection with the bacteria we can modify the environment at will, to an extent that is far beyond our power in regard to higher organisms; we can alter the media of growth, can make these more or less nutritious, can make them acid, neutral, or alkaline; can alter the superincumbent air to such an extent that if desired we can in many cases bring about growth in vacuo or in an inert atmosphere of hydrogen or carbonic acid; we can add or remove this or that normal or abnormal foodstuff to or from the medium of growth, or can add salts and antiseptics and observe the effects upon the microbes studied; we can observe the mutual effects of concurrent growth of two or more forms, and the effects which the products of growth of a microbe exert upon it, or again can modify the temperature within the widest limits. With no other forms of life are such extraordinarily diverse modifications of environment possible.

I propose now to set forth in regular order the facts at our disposal with regard to the production and existence of variations of microbes. To give all is, from their very extent, impossible in this connection, but sufficient may be given to show very clearly that this variation is far

* "Essays upon Heredity." Oxford, 1889, p. 278.

more widespread than is generally supposed, and that we can safely attribute to this variability many of the difficulties already mentioned met with in the clinical study of infective disease.

It is unnecessary for me to enter into the earlier history of the subject; with the controversy that raged around "polymorphism" and round Nägeli's theory as to variability[1] for at the time that these were most discussed the subject was not ripe for treatment. The methods of investigation were imperfect, pure growths could not with certainty be obtained, and consequently the arguments advanced *pro* and *contra* rested upon insecure bases. Still Lankester's observations on the change of form of the Clathrocystis, or Beggiatoa, Roseo-persicina[2] and later Kurth's "Observations on the Bacillus Zopfii"[3] have stood the test of time and may be said to have laid a solid foundation upon which rests our present knowledge of variation among microbes.

TRANSIENT VARIABILITY.

We possess now such a mass of separate observations upon transient variability of bacteria that it is difficult to know how much to say on this subject here, how much to leave unsaid. Every one who of late years has studied bacteriology must have come across numerous cases of this nature. Every one admits that according to the age of a growth, according to the nature of the medium of growth, changes in form and properties are common. They may be slight but, if looked for, are almost sure to be found. I would proceed to mention cases in which such changes are very marked.

(1) *Change of Form.*—The Bacillus Zopfii,[3] for example, when grown on various nutrient media manifests itself at first in the form of long twisting tortuous filaments; at a later period these give place to chains of bacilli of fair size, and eventually in an old growth the bacilli are replaced by what some would consider as cocci, others as spores. We need not now concern ourselves about this controversy. The important point is that, transferred to a fresh "soil," the bacillary form, just like the spore or coccus form, develops into typical long convoluted threads.

Even more definite changes of shape are producible by slight alterations in the medium of growth. Take, for instance, the Eberth-Gaffky bacillus of enteric fever. This is a bacillus of fair size—three to five times as long as it is broad, with rounded ends, and thanks to the possession of long vibratile cilia along its sides, it is normally endowed with great rapidity of motion. It has further the property of resisting the action of fairly large amounts of carbolic acid in the medium of growth—a property which has been utilised by Vincent to separate it out from other microbes and to gain pure diagnostic cultures from the fæces of those affected by the disease or from contaminated water If beef broth be taken containing about 1 part of carbolic acid in 600, the Eberth-Gaffky bacillus, almost alone among the bacteria, will grow in it at a temperature of 42° C., rendering the broth turbid in the course of a few hours. But if a drop of this be examined, one finds not the characteristic motile bacillus, but shorter forms and often diplococci, and these are non-motile. In fact, there is an absolute want of resemblance to the type bacillus. Yet if a culture of these be made in the ordinary beef broth of the laboratory, devoid of carbolic acid, there is an almost immediate return to type.[4] But there is a much simpler method of gaining polymorphism in connection with this microbe. Employing a series of potatoes of diverse origin it is found that upon some the growths of the microbe are atypical not only in naked eye appearance but also under the microscope, developing into long filaments. This atypical growth has been commented upon by numerous observers, including Fränkel and Simmonds,[5] Buchner,[6] Schiller,[7] and Kamen.[8] Buchner's observations render it probable that this atypical growth is in close relationship to the known varying acidity of the potato.

Still more striking are the results obtained by Charrin in the case of the small bacillus of blue pus, which within the body of an infected animal, or grown in beef broth, is what may be termed a micro-bacillus—a small short bacillus, the individuals being often attached in pairs, end to end.

If β-naphthol be added to the beef broth in the proportions of 0·02 per cent, after forty-eight hours long straight bacilli are to be made out, broader than the type, and from three to five times as long. If, instead, alcohol be added (about four per cent), at the end of twenty-four hours the change is even more pronounced; besides large bacillary forms, similar to those last mentioned, there may be somewhat curved filaments from ten to fifteen times as long as the type, if not longer. If 0·015 per cent potassium bichromate be added, then, even after fifteen hours, one can make out long undulating filaments stretching across the whole field of the microscope. And if the growth be acted upon by 0·7 per cent boric acid for six days, then in place of the micro-bacillus we see "comma" forms, S-shaped forms and close spirals; while 0·1 per cent creasote, acting for some weeks, causes the production of micrococci and diplococci.

There could be no better example than this of the polymorphism induced by environment. In all these cases, however, we are not dealing with permanent variations. Return to "ordinary" media brings about in all a return to what may be styled "type." I say what may be styled "type," inasmuch as the broth, potatoes, and other materials employed by the bacteriologist are the common soils in and upon which can be grown most bacteria, but they are not of necessity their natural habitat, and when environment evidently plays so important a part in determining the shape of bacteria it is quite possible that what we consider as typical are, in many cases, not the usual (or primitive), but are acquired forms.

(2) *Changes in Pigment Production.*—But shape is far from being the only property of microbes that is modified by environment, and very striking results are to be gained by a study of those microbes, which, in the course of their growth, produce pigment—the chromogenic bacteria.[10] Of these, a very large number lose the faculty of pigment formation in the absence of oxygen, many again, when grown in the absence of diffuse light, and all, under the action of the direct rays of the summer sun. An alteration of the medium of culture has also its effect. Thus the bacillus ruber of Kiel (better known in this country as the "rouge de Kiel," the name under which our attention was first drawn to it by Laurent[11]), the micrococcus indicus, the microbacillus prodigiosus, and yet other forms which produce a red colouring matter vary in the intensity of their colour production according to the acidity or alkalinity of the medium of growth. The most beautiful example of temporary modifications of frequent production by alteration of environment is supplied in Gessard's very full study of the bacillus pyocyaneus.[12] Ordinary beef broth sown with this bacillus soon takes on a greenish-blue slightly fluorescent appearance, and the blue colour becomes more marked if there be a relatively large surface exposed to the air, or if at intervals the culture be shaken, and the pellicle of surface growth be made to sink to the bottom of the vessel. Evidently then there are at least two pigments produced, and Gessard succeeded in causing the independent development of either. For he found that upon solidified white of egg the bacillus gave rise to the bright green colour alone, while upon the agar-agar, to which glycerine and a fair quantity of peptone had been added, no green was developed, the medium rapidly becoming tinged throughout an intense deep blue. It may be added that the absence of free oxygen causes the development of a colourless growth. These observations, I may say, can be easily confirmed.[13]

(3) *Modifications of Ferment Production.*—Similarly modifications can be induced in the ferment production of microbes. Thus many micro-organisms, which elaborate a ferment capable of liquefying gelatine, do not manifest that power if glycerine be added to the medium of growth, or (from a different cause) lose that power, as Cartwright Wood has shown[15] in the case of the Cholera-spirillum, of the bacillus prodigiosus and the micrococcus indicus, if minute quantities of carbolic acid be added to the culture medium. Again, as indicated by Lauder Brunton and Macfadyean,[14] the bacteria which form a peptonising enzyme on proteid soil can also produce a diastatic enzyme on carbohydrate soil.

(4) *Modifications of Pathogeneity.*—With regard to the temporary loss of pathogenic power—of virulence—the case is rather different. It

*

would seem to be most difficult to bring about a loss of virulence that does not tend to be permanent. That is to say, very special methods have to be employed whose action is exerted upon more than one "generation" of the microbes, such as passage through a series of animals less and less susceptible to the invasion of the given microbe in order to exalt the virulence to its former intensity. Indeed there are not a few micro-organisms, like the pneumococcus of Talamon-Fränkel, whose virulence can only be retained outside the body by constant removal to fresh media; others, like the typhoid bacillus, which according to Chantemesse and Widal[16] are so susceptible that a loss of virulence is almost immediate. This, after all, is only what is to be expected, if, as seems most probable, the pathogenic property is that which has been latest acquired. A late acquisition is most easily altered, and the tendency to re-acquirement of the same is least evident. Intensification of pathogenic properties can, however, be induced by other means than by passage through animals; Hueppe's observations would seem to show that the Bacillus Choleræ Asiaticæ grown anærobically gains in virulence.[29]

This principle—that it is difficult to stamp a modification firmly upon a species of bacteria, as, indeed, upon any animal or vegetable species—is exemplified throughout the long series of attempts that have been made to produce new races of micro-organisms, and now, when I proceed to recount some of the more successful of these attempts the principle will be seen to be constantly in evidence.

MODIFICATIONS OF LONGER DURATION.

From the transient modifications which I have noted above it is easy to pass to a series of instances in which the return to the normal only follows after an increasing number of generations, in which numerous generations retain the acquired characteristics, and the original properties only gradually reassert themselves. Take, for example, almost any of the chromogenic bacteria. If a minute quantity of a high-coloured typical growth be removed and spread over the cut surface of a sterilised potato it will be found that the individual colonies which result present differences in pigment production; the majority reproduce the type, but some are even deeper in colour than the original, others paler, others colourless. If from one of these colourless colonies fresh potato growths be made, a large number of coloured colonies develop, but also a large number of colourless, and the more frequently this selective process is repeated the greater becomes the proportion of the resulting colourless colonies. Yet even after a very great number of growths made in this way there is always a tendency for certain individuals and their progeny to revert to type. Slight alterations in the nutrition of the individuals probably induced the first differences in pigment production, and the induced modifications tend to reproduce themselves through many "generations."[9–13]

The same occurs if a culture of one of these non-pathogenic forms be heated for a few minutes to a point approaching that at which immediate death of the special microbe is brought about. After this treatment there may be numerous "generations" produced, incapable of pigment production. Illustrating this and the preceding order of appearances, I showed a series of specimens at the Nottingham meeting of the British Medical Association in July. The series included cultures of the Bacillus ruber, Plymouth; Bacillus ruber, Kiel; Microbacillus prodigiosus, Bacillus indicus and Sarcina erythromyxa. To yet more marked effects of high temperature I shall revert at a later period.

Again, old cultures of most micro-organisms become attenuated, and although these attenuated forms still propagate themselves, it may be weeks—that is to say, almost countless "generations"—before there is a return to primitive appearance and primitive properties. Thus old growths of Koch's Cholera spirillum,[15] or the Finkler-Prior spirillum of Cholera nostras;[17] or, again, of the Vibrio Metchnikovi[18] yield colonies which have an absent or greatly reduced power of liquefying gelatine, and only very slowly and under favourable conditions does this power manifest itself once more. From old cultures of the Bacillus pyocaneus, the Pink torula and many more chromogenic microbes, I find, in common with other observers, that colourless or faintly coloured growths are obtainable, which for long show no reversion to "type."

It would seem that without there being necessarily any attenuation there may be modifications persisting for long periods. It has been noted by many observers that for some considerable period after Staphylococcus pyogenes aureus has been separated out of pus and cultivated, the colonies are colourless, and that only eventually may the golden yellow pigment become produced. During the last long vacation I was a victim to this order of affairs, for having obtained a micrococcus from some pus gained from a case at the Addenbrooke's Hospital, I made two successive cultures of the pure growth during the course of a week, and the second of these I employed to distribute to the bacteriological class, as the Staphylococcus pyogenes albus. The cultures made by the class developed the golden-yellow pigment, and proved that we were dealing, not with the "albus," but with the "aureus." It may be added that, save for colour difference, the two forms are indistinguishable.

THE PRODUCTION OF RACES.

From such cases as these we can pass on almost imperceptibly to the development of what may be termed races. I will presently discuss the qualifications that must be applied in employing this expression. For immediate purposes it is enough to state that a slight modification of environment acting over a sufficient number of "generations"—that is to say, acting for a sufficiently long period—or a powerful stimulus applied temporarily to the individual bacteria of one "generation," may lead to modification of the properties of any species which, so far as we can see, are permanent so long as the microbes so acted upon are cultivated under ordinary conditions upon the usual media of bacteriological research.

To make this statement perfectly clear, I may give an example. The Bacillus ruber of Kiel grows well upon a piece of sterilised potato at the ordinary temperature (15—25° C.), the culture becoming a deep crimson red. If now the culture be kept at a temperature of 37° C. for two days, and a new piece of potato be inoculated from this and kept another two days at the same temperature, and a series of six to ten cultures be made thus; or if, on the other hand, a potato culture be exposed to a temperature of 55° to 57° C. for a few minutes, in either case fresh cultures made in series upon potato at the ordinary temperature may for months remain absolutely colourless.

It is scarcely necessary to say that the classical example of this order of phenomena is to be found in the remarkable observations of Pasteur, Chamberland, and Roux upon the attenuation of the anthrax bacillus.[19] The anthrax bacillus may be kept (in broth) at a temperature of 42·5° to 43° C. for a week or more without there being any noticeable diminution of the virulence of the growth. But after this the virulence slowly and steadily diminishes. Cultures made under ordinary conditions from a growth treated thus for twelve days will no longer, when inoculated, cause the fatal disease in sheep. After 31 days the cultures are so feeble that the very susceptible guinea-pigs survive, and mice alone are killed. In 40 days or so the bacilli subjected to the abovementioned temperature are enfeebled to the point of death, and no further cultures from the original broth are possible. What is more, each successive growth obtained daily after the eighth day from a culture subjected to the Pasteur process, if periodically resown, under ordinary conditions (in alkaline beef broth at 35° to 37° C), retains permanently—for months and years—the grade of diminished virulence that had been impressed upon the original culture.

Several other methods have been suggested for attenuating the anthrax bacillus, all of them possessing the advantage of being more expeditious[20–23] but all, save one, labour under the disadvantage that the lowering of virulence produced is unstable, and upon continued cultivation the successive growths vary and are liable to regain the pathogenic property of the original culture. The exception is Roux's method[23] in which, by the addition of minute quantities of potassium bichromate or carbolic acid to the culture medium, a race is, after a shor time, developed, which has lost the power of spore formation. According to the duration of the action of either antiseptic, and the amount of either which is present, so are the daughter cultures of particular degrees of virulence. These asporogenous races appear to retain their characters indefinitely under normal bacteriological conditions.

Here again it must be pointed out that "permanent" races are by no means necessarily attenuated races. Starting from a culture of the bacillus anthracis, so weak that only young mice are affected, it is possible, by passage of the virus through a series of animals whose resistance to the disease is in an ascending scale, to gain a series of races of gradually increasing virulence, and as Malm has shown[24] it is possible to pass beyond the virulence of the type and obtain a race that will kill not only sheep but the very refractory dog.*

Another example to the same effect may be gained from Pasteur and Thullier's researches into swine erysipelas (Rouget des porcs, or Rothlauf).[25] This fatal and most infectious disease is caused by a minute bacillus, first isolated by Löffler. Rabbits also succumb to the malady, but not so very readily. It is found that passage through these latter animals causes the virus steadily to augment in strength until a stage is reached most fatal to rabbits, but cultures obtained at this stage induce but a transient illness in pigs, which consequently can be inoculated or "vaccinated" against the disease. Here exaltation of virulence for one animal and attenuation of virulence for another go hand in hand. (Similar attenuation for the pig can be produced by keeping cultures at 37°C for a long period.)

Equally instructive cases may be gleaned from researches upon non-pathogenic micro-organisms. Let me take first the Microbacillus prodigiosus. It was found by Wasserzug[26] that the addition of antiseptics to the medium of culture, in quantities sufficient to retard growth, led not only to the loss of colour production, but also to accompanying remarkable changes in form; some individuals become much elongated, others develop into actual spirilla. This polymorphism and its extent depend (as I have already shown in the case of the Bacillus pyocyaneus) upon the composition of the medium. Upon returning such altered microbes back to the potato there is a gradual return to the original state. Yet it is possible by slight modification of this method to produce permanent races. Taking beef broth to which tartaric acid has been added,

* I may here call attention to a statement in Professor C. Fränkel's "Bakterlenkunde" (edition of 1891, p. 177) "Es ist . . bisher noch nicht geglückt, eine dauernde Verstärkung der Bakterien, eine haltbare zunahme ihrer natürlichen Virulenz herbeizuführen, ... auf Thiere. . eine raschere und andere Wirkungsweise an den Tag zu legen, als sonst an ihnen bemerkt wurde." While these statements, in face of French researches, were, to say the least, already very debateable a year ago, they certainly now cannot be regarded as other than quite incorrect.

**

Wasserzug found that when the culture was a day or two old there was a great development of spirilla. Later, when through the production of trimethylami the medium became alkaline, the spirilla ceased to be developed; there was a return to the coccus or shortened bacillary form. If now he did not permit the production of the alkaline trimethylamin, but made new acid growths before the cultures were more than forty-eight hours old, he discovered that the longer he persevered with his series of cultures the longer the microbe took when grown upon potato to return to the coccus form, until finally by such acid growth and subsequent heating to 50°C. for a few minutes, all that he could obtain upon sowing on potatoes was, not a micrococcus, but a long bacillus—a permanent race of such.

Or let us return again to the Bacillus pyocyaneus which, though pathogenic, may be discussed along with other chromogenic forms.

By placing his modifications, to which I have already referred, under special conditions of heat, etc., Gessard found that he could obtain permanent races upon ordinary beef broth, one giving the green fluorescent pigment alone, another the blue pyocyanin, another colourless.[12] The same observer, studying the bacillus of blue milk (Bacillus cyanogenus or syncyanus) found that by passages through egg albumen he could obtain a race giving a deep pigment (which becomes red with alkalies, blue with acids), another race which is only fluorescent, gained from cultures that are some months old, and a third colourless race, this last, either by taking extremely old cultures or by warming a recent culture to a temperature which does not cause death.

Similarly Laurent,[11] working with the Bacillus ruber from Kiel (Bacillus rouge de Kiel), found that exposure of a growth to bright sunlight for three hours gave colonies which with but few exceptions were colourless, and though with further successive cultivations there was manifested a tendency for many of the latter colonies to revert to type and develop red pigment, yet by careful selection he was able to gain an absolutely decolourised race which remained colourless, that is, upon agar-agar and in broth at a temperature of 25° to 35° C., a temperature and media at which and in which the original growth always shows a strong reddish violet tint. On potatoes at the same temperature he was able to make thirty-two successive cultures extending over a year, and in these not the least trace of colouration ever appeared.

These facts, if alone they were all that we had to consider, would fully justify the statement that it is possible to evolve artificially species or sub-species of bacteria. But there are other facts which must of necessity be taken into account before we can arrive at any definite conclusion—facts which so far as I know apply at present to micro-organisms alone, though everything points to the belief that they are operative, and should be considered, in discussing the development of new species and sub-species among higher organisms.

In cultivating the bacteria we are confronted with the fact, that successful culture necessitates the employment of special media of growth. We have to sow the microbes in fluid or solid substances whose composition depends upon the mode of life of the special form studied. And thus there is and can be no common soil in which all forms propagate themselves with equal facility. We are deprived of what I may term a common base. We cannot, with certainty, say that such and such a form, which we have separated out, is a distinct species, inasmuch as grown upon a common normal soil it presents permanent characteristics. The most that we can do is to approach, as near as possible, to the formation of such a common soil. Thus for many pathogenic and most other bacteria we find that slightly alkaline beef-broth, and beef-broth that has been rendered solid by the addition of gelatine or agar-agar, are media in which growth occurs freely, and in which, certain elementary precautions being taken, a given micro-organism retains its characters for countless "generations," retains them so completely that, in the case of the majority of pathogenic microbes a series of successive cultures, if made with due precautions and sufficient frequency, will yield microbes which will all produce typical symptoms when inoculated into a series of rabbits, guinea-pigs, or other animals, the microbes of the last of the series of successive cultures possessing the same degree of virulence as the first of the series.

Where this is the case, are we justified in stating that the species is permanent? Upon first consideration one is inclined to say that undoubtedly we are, yet further thought brings in doubt, for this is far from being all. There is another and remarkable order of appearances to be considered, which can be exemplified from every case that I have given of the establishment of permanent races.

Take, for instance, the races of anthrax bacilli produced by Pasteur's method. These, undoubtedly, when kept upon our approximation to a common soil, namely, upon beef-broth, preserve a well-defined permanency. But select any one of these, and pass it through a series of animals of increasing refractoriness to the disease, and there is evolved a race growing equally well upon the beef-broth, whose virulence has been greatly increased. Or take Gessard's "permanent" modifications of the Bacillus pyocyaneus and the Bacillus cyanogenus respectively; grow any of the former upon the rich culture medium formed of agar-agar, glycerine, and peptone, and all develop equally into a race producing large quantities of an intense blue pigment; grow any of the latter upon broth containing 2 per cent of glucose, and all give rise to the specific blue pigment of the typical bacillus. Again, take Laurent's colourless race of the bacillus "Rouge de Kiol," and place it upon potatoes at a temperature of about 18° instead of 25° to 35° C., and the coloured form reappears.

Or to epitomise: It is possible with bacteria to bring about modifications which persist when a return is made to the ordinary media of the bacteriologist (whether these be only our approximation to the normal—beef-broth, potatoes, etc.—or whether they be the bodies of one or other species of animal—mice, sheep, etc.)—so that now cultivated upon these "ordinary" media, the modification preserves its characteristics, and remains markedly different from the initial or type growth upon the same media. And here we have a distinction between the races of bacteria and the races of animals under domesticity, which, returned to the wild state, are said to revert to type. Now with the bacteria, where such races have been developed, reversion to type may still be brought about by a further process, namely, by *a further alteration of environment.* I know of no case quite analogous to this among the higher animals.

It is only within strictly limited conditions of environment that the characteristics of the usual form or of a race can be preserved; overstep these limits, make certain alterations in the environment, and type and race alike are liable to vary, although the liability is perhaps the greater for the race to approximate towards the type than for the type to produce well-marked races. The only cases that I can call to mind where this law apparently does not hold good are both of modifications of the anthrax bacillus. It is possible so to attenuate the anthrax bacillus that a race is developed which is absolutely non-pathogenic even for the most susceptible of small rodents, and so far no efforts have succeeded in re-establishing the virulence of such attenuated forms. Roux's asporogenous race also would seem thus far to remain asporogenous under all conditions, although variations may be induced in its virulence. Even these cases, however, will probably be found eventually not to be exceptions to the rule.

For these reasons I have throughout spoken of types and races rather than of species and subspecies or varieties, for by employing the more uncommon terms in this connection I have attempted to guard myself against appearing to hold that among the bacteria there is anything beyond a relative permanency. A study of the observations that have been made up to the present time does *not* lead to the conclusion that new species are readily developed—all that it clearly indicates is that among the bacteria our employment of this term "species" must be elastic—the limit to which the individual may depart from the type (and from which it may again revert to type), is very far removed. But granting freely that not one of the cases here described records the development of a new species, this much at least must be admitted, namely, that the longer the action of any one factor upon the bacterial growth, the longer the time requisite to ensure a return to the typical condition; the stronger the impress left upon any individual microbe, the longer again the time requisite to ensure a similar return. If, therefore, within the relatively short period of experiment changes so marked, changes persisting through so many generations or cell multiplications can be brought about, surely it is not difficult to comprehend changes in the natural world, whose action is sufficiently great and is prolonged through a sufficiently long period to produce races of even greater permanency, races which might be termed new species.

ON NATURAL RACES.

Now apart from these results of experiments, we possess already a series of data whose explanation is easiest on the supposition that we are dealing not with forms that are wholly distinct and of separate origin, but with allied forms and natural races. Perhaps the best example is one that has been extensively discussed. Fehleisen's streptococcus erysipelatosus, the microbe causing erysipelas, is, in microscopic appearance and in mode of growth, undistinguishable from the streptococcus which can be isolated from many boils and cases of abscess formation. Yet certainly the two micro-organisms differ in pathogeneity, differ in the symptoms they induce when isolated. Are we to say that here we are dealing with two distinct species? A few years ago all bacteriologists answered this question in the affirmative, but now most would reply in the negative, although some, among whom may be included Crookshank,[27] still adhere to the old view. The observations of Eugen Fränkel would seem to indicate that truly we are dealing with what are only races[28]. Fränkel obtained from a case of "universal peritonitis" a pure growth of streptococcus. There could be here no question of erysipelas. Taking some of a fifth culture of this, five months after it had been isolated, he inoculated it into a rabbit's ear and produced an exquisite bullous erysipelas, with streptococci in the lymphatics, identical with human erysipelas. The same inoculated into the peritoneal cavity gave rise to a fibrinous or fibrino-purulent peritonitis. From this it is evident that the difference between erysipelas and pyæmia, in its various forms, depends on mode and locality of infection and partly on the quantity of virus, on the individual, and lastly, on differences in the intensity of virulence of the races of streptococcus.* It is interesting to note how, in clinical practice, one observes this conclusion borne out; to observe the succession of cases passing through ordinary erysipelas and acute

* A paper by Behring[33] published during the last month, confirms this view of the identity of the pathogenic streptococci, and shows that an animal rendered immune to one of the various races of these (*vide* Lingelsheim[34]), is now immune to all other pathogenic streptococci.

lymphangitis,[30] bullous and phlegmonous erysipelas, and acute pyæmia. Indeed, one is driven to the same conclusion with reference to the streptococcus pyogenes that Levy arrived at after several years' work at pyogenic micro-organisms in general.[31] Levy denies that anything can be prognosed from the nature of a microbe causing suppuration. "The prognosis of the process whose origin is referable to a micro-organism, depends upon the virulence of the same, and the degree of virulence of pyogenic micro-organisms is subject to extraordinary change."

It may be that similar changes in virulence will explain the phenomena displayed in the case of swine erysipelas and mouse septicæmia, cholera, and the Odessa fowl disease respectively. The micro-organisms of the first pair of diseases are in appearances, size, and method of growth scarce to be distinguished; but whereas the one form produces a fatal disease in pigs, the other has no action whatsoever upon these animals; rabbits also are susceptible to swine erysipelas, while they are said not to be affected by mouse septicæmia. Still it is worthy of notice that Löffler has occasionally obtained fatal results in rabbits with inoculations of the bacillus of the latter disease, and that the bacilli of both diseases show themselves peculiarly fatal to white mice, producing similar effects. Quite recently, Lorenz[32] has shown that pigs inoculated with cultures of the bacillus of mouse septicæmia are protected against swine erysipelas, and has described a third form, causing an eruptive disease in pigs, which he terms "Backsteinblattern." The bacillus of this, in mode of growth and properties, is intermediate between the other two, and of these three any one, either virulent or properly attenuated, confers immunity against the other two.

So again with the second pair of diseases. It is impossible to distinguish with certainty a preparation of the vibrio Metchnikovi from one of Koch's cholera spirillum, and the differences in the mode of growth of the two are not greater than may be determined between two cultures of the cholera spirillum of different origin. Yet vibrio Metschnikovi is pathogenic for pigeons and fowls and only affects guinea-pigs (which are susceptible to inoculations of the cholera spirillum) when inoculated in relatively large quantities. According to Gamaleïa[33] injections of the vibrio Metschnikovi render guinea-pigs immune to cholera. Pfeiffer denies this, though Gamaleïa has reiterated the statement, and ascribes Pfeiffer's failure to gain immunity to the fact that he employed attenuated cultures. It must, however, be admitted that Gamaleïa's statement still awaits confirmation, and, for the present, it is better to look upon the series of microbes which includes Koch's spirillum of Asiatic cholera, the Finkler-Prior spirillum of cholera nostras, Deneke's S. tyrogenum, Miller's spirillum obtained from the mouth, and the vibrio Metchnikovi as a group of very closely allied species.

Similarly so high an authority as Professor Hueppe would class together into one group, as the bacteria of hæmorrhagic septicæmia, a large number of bacteria which microscopically are undistinguishable, whose ends stain more deeply than the central region, whose growth upon bacteriological media is similar, and which further are identical in the appearance of the individual colonies. C. Fraenkel would separate these into two groups, one including the bacillus of ferret plague (Eberth and Schimmelbusch), the bacillus of swine plague (Billings), that of hog cholera (Salmon), that of Danish swine plague (Selander), and the bacillus of the German "schweinepest." All these are motile and ciliated. The other group contains forms that are identical, or almost identical, namely, Löffler's bacillus of chicken cholera, Schutz's swine plague bacillus, Cornil's bacillus of duck cholera, Gaffky's microbe of rabbit septicæmia, and Kitt and Hueppe's bacterium of "Wildseuche" (deer plague). All these only differ in regard to the animals which they specially affect.[35]

Here, too, I would mention a most interesting observation of Hueppe and Cartwright Wood. These observers gained from ordinary earth a harmless saprophytic bacillus, in appearance and mode of growth resembling closely the bacillus anthracis. Inoculated into mice, which are of all animals the most susceptible to anthrax, pure growths of the earth bacillus induced immunity against this very fatal disease.[36]

ON THE OBSERVED MODIFICATIONS OF PATHOGENIC MICROBES.

There is, however, another and perhaps more satisfactory way of approaching this subject of the occurrence of natural races. I refer to the actual differences in mode of growth and properties that are to be distinguished in cultures obtained from diverse typical cases of any given disease. This subject has not been worked out so fully as it deserves, nevertheless certain very interesting observations have been made. Thus the Talamon-Fränkel diplococcus of acute croupous pneumonia has been studied by Banti.[39] Banti describes four varieties of the species diplococcus lanceolatus capsulatus. No. 1 is the typical form described by Fränkel and Weichselbaum. No. 2 is identical in form and culture, but causes a "Diplococcus septicæmia" in rabbits, with small spleen and destruction of the red corpuscles. No. 3 is similarly identical, but produces a septicæmia with moderate enlargement of spleen and diffusion of hæmoglobin, and No. 4 only differs from the rest in that its virulence outside the body disappears even more rapidly than is the case with the other forms; it produces a mild septicæmia associated with albuminuria, and the animals all recover. All were obtained originally from typical cases of pneumonia, and while in the years 1886, 1887, and 1890, the type form was alone gained from almost every case of pneumonia—and these were of a benign nature—in 1888 and 1889 the type No. 1 was never isolated, but the other varieties were present, and the cases were severe.*

Foà[41] has shown that by inoculation variation may be induced in the diplococcus. If from a rabbit that has died of inoculation pneumonia two rabbits be inoculated, one from the fresh fibrinous pneumonic exudation, the other from the cerebro-spinal fluid, it is found that the disease differs in the two. The former shows inflammatory œdema of the skin, the latter none. So, too, if the diplococcus be grown for twenty-four hours anærobically the latter type is produced and its properties persist, although the original virulence and characters may be restored by simultaneous inoculation of this form along with the staphylococcus pyogenes aureus and the proteus vulgaris. These studies of Foà find their counterpart in Banti's researches into acute primary meningitis.[42] From two cases of this disease Banti isolated a diplococcus not to be distinguished from that of Talamon-Fränkel, except that it rapidly lost its virulence both inside and outside the body, so that when the meningeal exudation was inoculated into a rabbit, and from this a further series of passages was made, the sixth rabbit had a mild non-fatal disease.

In the case of typhoid, Babes[43] has made a long series of observations, and though he handles the subject with what for him is extreme caution it is difficult to arrive at any other conclusion than that the facts he represents can only be satisfactorily accounted for on the assumption that, granting there be a specific micro-organism for typhoid, and granting his results to be reliable, this micro-organism is capable of modifications so definite that many races are developed. Babes made numerous cultures from twelve typhoid corpses, and while in nearly every case he found the typical form, in every case he discovered also varieties. In all he describes 18 of these, all presenting some slight differences. These were sufficiently permanent to permit him to declare that he never saw one revert to the original form (as determined by an original culture from Berlin), although in many there was a tendency towards the loss of characteristics. While acknowledging that these varieties, with rare exceptions, produce in small animals lesions similar to those due to the typical bacillus, he concludes that they are not specific, and that typical and atypical belong to a group possessing similar conditions of existence. He notes also that the baccillus coli communis possesses many natural varieties. Despite this various cautious conclusion, Gaffky

* An observation of Nikiforoff's may be quoted in this connection[40]. From the lung of an influenza patient he obtained a microbe, identical with the diplococcus pneumoniæ in every respect save that it preserved its virulence for longer periods, and was fully virulent for mice, while rabbits were completely refractory, whereas the true diplococcus is virulent for both mice and rabbits.

(who with Eberth shares the honour of having discovered the typhoid bacillus) criticised Babes severely, and suggested that all his varieties were due to secondary "Einwanderung."[44] To this Babes replied[45] by showing that if so the varieties had wandered in during life, for his autopsies were made upon the fresh corpse very few hours after death. Surely rather than multiply species to the enormous extent that these conclusions would demand it is simpler, as it is more inherently probable, to hold these varieties to be races and of a common origin—races, it may be, produced by the peculiar conditions of the contest between organism and microbe. At the same time I am not prepared to go as far as Arloing[46] who declares that the bacillus of typhoid can be developed from the bacillus coli communis in the presence of fermenting fæcal matter.

The subject of the bacillus of typhoid cannot be passed over without mentioning also Cassedebat's important paper.[47] The relation of epidemics of enteric fever to the water supply has been so clearly traced that all that was wanting to afford an absolute proof of the relationship was the demonstration of the presence of the specific bacillus in the suspected sources. And this demonstration would seem to have been made by the methods of Vincent, Rodet, Kitasato, Chantemesse and Widal, Thoinot, and yet others. But Cassedebat, working at Marseilles where enteric fever is almost endemic, states that in the water supply—the Durance—he was unable to discover the typical bacillus, although in its place he determined three forms all resembling the bacillus of typhoid in their mode of growth upon potato (which is very characteristic), and in the form of the colonies upon agar-agar plates, all equally polymorphous, and staining with difficulty, all possessing the same movements of translation and oscillation, and giving much the same growths upon puncture of gelatine, while in even so minute a characteristic as the arrangement of the cilia, Löffler's method showed no difference between No. 1 and the type. (It is not stated how the others appeared under this treatment). But there were differences of rate of growth on potatoes, in gelatine, potato-juice-gelatine, and broth, differences also in the intensity of colour of potato cultures, differences in the effect of the bacilli upon media to which aniline dyes had been added, and the result on inoculations, which were far from complete, show that on the whole No. 1 was less virulent for mice than in the type Eberth-Gaffky bacillus. From these differences Cassedebat concludes that he is dealing with pseudo-typhoid forms, and that the results of the bacteriological examination of water must be received with very great caution, if they are to be accepted at all. I have already shown that the Eberth-Gaffky bacillus is capable of very considerable modifications, is most susceptible to change of medium, etc., and here, again, rather than accept Cassedebat's conclusions, I would hold that the more satisfactory explanation of his facts is that he dealt with races—natural and fairly permanent races—modifications of the type bacillus of typhoid.

Turning now to cholera and its spirillum, it is already a well-known fact—to which attention was, I believe, first called by Zäslein[48]—that cultures of the spirillum derived from simultaneous epidemics in different localities present recognisable differences—differences in tint, in rate of growth, in power of liquefying gelatine, etc. So there are those who declare that from the appearance of a culture they can state its origin, whether from Cairo or from Berlin, from Naples or Massowah, from Palermo or Marseilles. It has, however, been left to Surgeon-Major Cunningham to give the most remarkable example of these differences.[49] At the beginning of 1890, three of the principal hospitals in Calcutta contained cases of cholera, not differing from one another in symptoms or virulence, all giving abundance of cultivable spirilla, but these not of one, but of three "species." Later, other cases were observed, in all sixteen, and from these sixteen ten "species" were obtained α to κ. In only four of the cases was the form present, even doubtfully, that described by Koch (Cunningham, bacillus α). In some, three different comma bacilli were present at the same time.

The forms described by Cunningham may be divided into two groups. The first contains only one which does not liquefy gelatine, does not show interstitial growth in agar-agar, does not develop "cholera red" on the addition of acids, and morphologically is distinguished by its large size and great variability of form and curvature. This, I feel inclined to consider as a truly different species. The other group contains all the other nine, which liquefy gelatine, are capable of interstitial growth, give the "cholera red" reaction, but all show fairly permanent differences in the rate of liquefaction of gelatine. In six the rate is distinctly rapid, in the rest it is slow. In one, growth upon potato is luxuriant, in others there is rare acclimatisation to this medium, in others again acclimatisation has never shown itself. Here, then, one working where cholera is endemic, and one who is a most capable observer, who had been with Koch when he studied the disease in India, is so affected by the general view that species are constant and distinct, that the only conclusion that he can draw from these facts is that "whilst denying that the primary cause of cholera is represented by any species of intestinal schizomycete which has yet been discovered" it may be "that those whose development is favoured by the existence of the choleraic condition may exert an important influence on the ultimate outcome of individual cases of the disease." If we accept the view that the spirillum is specific I do not see any other conclusion from these observations than that here is another well-marked instance of the production of allied races.

Only within the last few days news has come from St. Petersburg that the spirillum obtained from patients affected in the cholera epidemic now raging, presents marked differences in size, etc., distinguishing it from Koch's original form ; while from the Institute Pasteur I hear that the microbe, isolated from some of the doubtful cases in Paris, presented no greater departure from type than is to be seen in many of the growths obtained from indubitable cases of cholera.

I might enter into the discussion of the relationship between human, bovine, and avian tuberculosis, or again into the differences to be made out between the bacilli of pulmonary and surgical tuberculosis (lupus, tuberculosis of bone and joints, and scrofula), but though much has been done on these subjects there is a want of well confirmed results, and the subject is still too chaotic for safe treatment. I will conclude this relation of the occurrence of natural races with a reference to Roux and Yersin's remarkable and admirable work upon diphtheria.[50] Apart from proving that the bacillus isolated by Löffler[51] is specific, by showing that the sterilised medium of growth inoculated into animals will induce the characteristic symptoms of the disease (including the slowly produced paralyses), these observers made careful studies of the pathogenic qualities of the microbe. They found that, according to their origin, some cultures would kill guinea-pigs in twenty-four hours, some in sixty, some in only three or four days, or even after a longer interval, and they turned their attention to a form or forms, that had been described by Löffler as the bacillus pseudo-diphthericus. Löffler found this in the false membranes along with the virulent micro-organism, and pointed out that it is practically undistinguishable from this last, save that it has no toxic effect upon animals. Hoffmann found it also in the pharyngeal mucous membrane of cases of scarlatina and measles. Its discoverer considered it a separate species, and in this view most observers joined, though Klein and Flügge admitted that it was an attenuated form. The colonies on coagulated blood serum are identical with those of the diphtherial bacillus; like that, it grows rapidly at a temperature of 33° to 35° C. ; microscopically, the two are the same, the staining reactions are similar, and growth in alkaline beef broth is characterised by the same production of acidity followed by increasing alkalinity. The differences are, that it is often shorter when grown on blood serum, that the cultures on broth are more abundant, and so are those on agar-agar. It still grows at 20° to 22° C., a temperature at which the virulent bacillus ceases to proliferate, and the alterations in alkalinity and acidity of both cultures proceed more rapidly.

This form is very rare in malignant cases of diphtheria—it is more abundant in benign, and becomes increasingly common as severe diphtheria progresses towards cure. But as I have already indicated, it is to be discovered in non-diphtherial cases. At the Hôpital des Enfants Malades, Paris, from the pharyngeal mucous membrane of 45 children not affected with the disease it was obtained in 15 cases, and of 59 healthy school children at Caen, in a school where for long there had been no diphtheria, no less than 26 gave growths of this form, though it must be confessed that the colonies were few and far between. Inoculation into guinea-pigs and rabbits never led to fatal results, and yet differences were observable. Some guinea-pigs manifested a very considerable œdema at the point of inoculation, in others a slight œdema was to be seen, others presented no sign of even local disturbance. It is noteworthy that the most marked œdema was developed where cultures were employed whose origin was from cases of measles.

Œdema at the point of inoculation is one of the characteristic lesions when virulent diphtheria bacilli are injected, and Roux and Yersin found that if they attenuated virulent cultures either by the action of air and high temperature, or again, if they preserved dried-up false membranes for five months in a cool place and in the dark, they gained colonies, some of which caused no œdema, others a little, one alone caused marked œdema. Such attenuated bacilli, like the bacillus pseudo-diphthericus, grew more abundantly at a lower temperature, and, like this, also rendered broth more rapidly alkaline. In fact, the only point distinguishing the two was that our observers succeeded in intensifying the virulence of the attenuated form, and failed to do this with the pseudo-diphtherial bacillus. In every other respect the proof appears conclusive that this bacillus pseudo-diphthericus is a natural race, or races, of the bacillus diphthericus, that it is no wise a separate species of independent origin.

What is more, Roux and Yersin suggest an explanation of the onset of diphtheria—of the natural intensification, that is, of the virus in man. Just as they found that from a case of measles presenting no sign of diphtheria proper they gained a race of the bacillus pseudo-diphthericus, which exhibited distinct, if slight, virulence, so have they noticed cases in which, through the access of an anginal attack, or of measles, diphtheria, which was recovering, gained fresh malignancy and became rapidly fatal.

There is another micro-organism frequently found in the mouth and saliva of healthy persons, namely, the Talamon-Fränkel diplococcus. May it not be that in both these cases a natural intensification of the virus is brought about by catarrhal and other affections of the upper respiratory and pharyngeal mucous membrane, and that to such intensification, rather than to the entry afresh of virulent micro-organisms, is to be ascribed the onset of these diseases?

I am well aware that in setting in order a series of facts and observations upon deviations from the normal the tendency is peculiarly strong to see in such deviations the normal, to see in the normal the exceptional, and it may be that in the preceding pages I have not dwelt with sufficient emphasis upon the fact that employing well recognised and standard methods the typical forms of bacterial growth are easily and most usually to be separated out from cases of disease. Still I shall feel that these pages have not been written in vain if I succeed in drawing increased attention to the fact that the bacteria are organisms acutely susceptible to changes in environment, that as a species they are far from presenting constant characteristics, and that to a variability which may impress itself upon a greater or less number of generations is to be ascribed, *in part*, the differences between successive epidemics, between the successive stages of one epidemic, and between individual cases of disease.

REFERENCES.

1. NÄGELI.—"Die niederen Pilze," Munich, 1877.
2. LANKESTER.—*Quart. Journ. Micr. Science*, XIII., 1873, p. 408.
3. KURTH.—*Botanische Zeitung*, 1883.
4. VINCENT.—*Comptes rendus de la Soc. de Biologie*, 1890, No. 5.
5. FRÄNKEL and SIMMONDS.—*Zeitschrift f. Hygiene*, II., 1887, p. 138.
6. BUCHNER.—*Centralblatt f. Bakteriologie*, IV., 1888, p. 353.
7. SCHILLER.—*Arbeitem aus dem Kais. Gesundheitsamb*, V.
8. PETRUSCHKY.—*Centralblatt f. Bakteriologie*, VI., 1889, p. 657.
9. CHARRIN.—"La maladie pyocyanique," Paris, Steinheil, 1889.
10. SCHOTTELIUS.—*Kölliker's Festschrift*, Leipzig, 1887.
11. LAURENT.—*Annales de l'Inst. Pasteur*, IV., 1890, p. 465.
12. GESSARD.—*Annales de l'Inst. Pasteur*, IV., 1891, p. 737; and V., 1891, p. 65.
13. ADAMI.—Meeting of East Anglian Branches of British Medical Association Cambridge, *Lancet*, July 24, 1891.
14. LAUDER BRUNTON and MACFADYEAN.—*Proceedings Royal Society*, XLVI., 1890, p. 542.
15. CARTWRIGHT WOOD.—*Proc. Royal Soc. Edin.*, XVI., 1889; and *Laboratory Report R. C. P. Edin.*, II., 1890, p. 253.
16. CHANTEMESSE et WIDAL.—*Annales de l'Inst. Pasteur*, II., 1888, p. 54.
17. FIRTSCH.—*Archiv. für Hygiene*, VIII., 1888, p. 369.
18. PFEIFFER (R.).—*Zeitschr. für Hygiene*, VII., 1889, p. 347.
19. PASTEUR, CHAMBERLAND, and ROUX.—*Comptes rendus*, XCII., 1881.
20. TOUSSAINT.—*Comptes rendus*, XCI., 1880.
21. CHAUVEAU.—*Comptes rendus*, XCVI., 1883, p. 678.
22. WOSSNESSENSKY.—*Comptes rendus*, XCVIII., 1884, p. 314.
23. ROUX.—*Annoles de l'Inst. Pasteur*, IV., 1890, p. 1.
24. MALM.—*Annales de l'Inst. Pasteur*, IV., 1890, p. 520.
25. PASTEUR and THULLIER.—*Comptes rendus*, XCVII., 1883, p. 1113.
26. WASSERZUG.—*Annales de l'Inst. Pasteur*, II., 1888, p. 75.
27. CROOKSHANK.—International Congress of Hygiene and Demography, August, 1891.
28. FRÄNKEL (EUG.)—*Centralb. f. Bakteriologie*, VI., 1889, p. 691.

29. HUEPPE.—*Berliner klin. Wochenschrift*, No. 9, 1890.
30. VERNEUIL and CLADO.—*Comptes rendus*, CVIII., 1889, p. 714.
31. LEVY.—*Arch. f. exp. Pathol. und Pharmak.*, XXIX., 1891, Parts 1 and 2.
32. LORENZ.—*Archiv f. wiss. u. prakt. Thierheilkunde*, XVIII., 1892, p. 38.
33. BEHRING.—*Centralblatt f. Bakteriol.*, XII., 1892, p. 192.
34. LINGELSHEIM.—*Zeitschrift f. Hygiene*, X., 1891, p. 331. (Gives literature of subject.)
35. GAMALEÏA.—*Annales de l'Inst. Pasteur*, III., 1889, p. 609.
36. GAMALEÏA.—*Annales de l'Inst. Pasteur*, IV., 1890, p. 330.
37. FRÄNKEL (C.).—*Grundriss der Bakterienkunde*, Berlin, 1891, p. 461.
38. HUEPPE and WOOD.—*Lancet*, December 7, 1889.
39. BANTI.—*Lo Sperimentale*, XLIV., 1890, pp. 349, 461, 573.
40. NIKIFOROFF.—*Zeitschr. f. Hygiene*, VIII., 1890, p. 531.
41. FOÀ.—International Medical Congress, Berlin, 1890 (Section of General Pathology).
42. BANTI.—*Lo Sperimentale*, XLIII., 1889, p. 138.
43. BABES (V.).—*Zeitschrift f. Hygiene*, IX., 1890, p. 323.
44. GAFFKY.—*Hygienische Rundschau*, 1891, No. 12.
45. BABES (V.).—*Centralb. f. Bakt.*, X., 1891, p. 281.
46. ARLOING.—International Congress of Hygiene and Demography, London, 1891.
47. CASSEDEBAT.—*Annales de l'Inst. Pasteur*, IV., 1890, p. 625.
48. ZÄSLEIN.—*Deutsche med. Wochenschrift*, 1888, Nos. 64 and 65.
49. CUNNINGHAM (D. D.).—"Scientific Memoirs by Medical Officers of the Army of India," Part VI., 1891, p. 1.
50. ROUX and YERSIN.—*Annales de l'Inst. Pasteur*, IV., 1890, p. 385.
51. LÖFFLER.—Congress of Military Surgeons, Berlin, 1887; and *Centralb. f. Bakt.*, II., 1887, p. 105.

The following papers may also be consulted:—

HANSEN (E. C.).—*Annales de Micrographie*, II., 1889, p. 214. ("On the Production of Varieties among the Saccharomycetes.")

METCHNIKOFF.—*Annales de l'Inst. Pasteur*, III., 1889, pp. 61 and 265. ("On a Remarkable 'Spiro Bacillus' [Polymorphic] Parasitic in Daphnia.")

WINOGRADSKY.—*Annales de l'Inst. Pasteur*, III., 1889, p. 244. ("On Polymorphism.")

HUEPPE.—*Berliner klin. Wochenschrift*, 1884 (see also No. 38). ("On the Relationship of Saprophytic to Pathogenic Bacteria.")

DELÉPINE (SH.).—*Transactions, Pathological Society*, London, 1891. ("Mutability of Pathogenic Aspergillus, as also of the "Characters of the B. Lepræ.")

UNNA.—*Deutsch. Naturforscherversammlung*, Halle, September, 1891, and *Centralblatt f. Bakt.*, XI., 1892, p. 638. ("Races of the Favus Fungus [Achorion Schonleinii"]).

JOHN HEYWOOD, EXCELSIOR PRINTING AND BOOKBINDING WORKS, MANCHESTER. [5,152 A

of

).

of

t.,

of

a

.")
ip

of

att
]).

2 A