THE
TRANSMUTATION OF BACTERIA

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BY

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"Ogni primaio aspetto ivi era casso:
due e nessun l' imagine perversa
parea,...
Così vid' io la settima zavorra
mutare e trasmutare; e qui mi scusi
la novità, se fior la penna abborra."

Dante: Inf. XXV.

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PREFACE

THIS essay is based upon notes and observations which I collected previous to the year 1913. It was only partly written when, in August 1914, I proceeded on active service. I was able, however, to complete it in the following summer while serving with a Field Ambulance in France, and in the autumn of the same year (1915) I submitted it in the form of a Dissertation for the degree of M.D. at the University of Cambridge.

The difficulties in carrying out work of this character while serving at the Front—remote from libraries and amidst "alarms and excursions" which break up one's scanty leisure —are sufficiently obvious and I trust may excuse some of its defects. Some valuable materials which I had hoped to utilise, including chapters on Viability and Agglutination Reactions, were buried by a shell explosion and could not be replaced.

The claims of Army work have also precluded any attempt on my part to bring the work up to date by reference to papers published since the beginning of the war. I particularly regret having learnt too late to include any mention of it in the following pages of the valuable research carried out by Dr Thiele and Dr Embleton on the part played by the body ferments in the pathogenicity of bacteria.

Though I have endeavoured to suppress all irrelevant matter, I am only too conscious of the discursiveness of this essay. The topic is one of absorbing interest and at every step one is tempted to digress. In the words of Dante, which I have quoted on the title page, "The novelty must be my excuse if my pen has wandered at all."

I have only touched the fringe of the subject. An inexperienced sailor in my "piccioletta barca," I have been tossed about in the breakers of this uncrossed sea. To others, better equipped by knowledge and training than I am to explore it, I would say—

"Metter potete ben per l' alto sale
vostro navigio,...
Quei gloriosi che passaro a Colco
non s' ammiraron, come voi farete,
quando Jason vider fatto bifolco."

(Dante: Par. II.)

S. G.-D.

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CHAPTER VII

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Pathogenicity is power to produce in certain animals certain symptoms and certain lesions—quite distinct from virulence and other characters. Generally regarded as more fixed than other characters—constitutes final appeal in doubtful cases, *e.g.* Hofmann's bacillus and Klebs-Loeffler bacillus—gonococcus and meningococcus—gonococcus does not cause meningitis nor meningococcus urethritis. Pathogenicity a variable character in all three aspects.

I. VARIATION IN KIND OF ANIMAL AFFECTED.

II. VARIATION IN SYMPTOMS CAUSED.

(1) Same organism causes different symptoms in different cases. Symptoms may depend upon organs affected—cf. lead poisoning—this determined by route of infection and vitality of organs—also by pathogenicity of organism—*e.g.* tubercle bacillus causes phthisis, osteitis, arthritis, lupus—unlike lead poisoning types remain distinct—skin rarely infected by tuberculous sputum—contrast between gonococcus and meningococcus no greater than between different strains of tubercle bacilli. Fallacy due to pre-existing disease, *e.g.* nephritis in cerebrospinal fever.

(2) Pathogenicity can be artificially modified, *e.g.* that of *B. anthracis* by ultra violet rays.

(3) During epidemic different cases exhibit different symptoms.

S. scarlatinae causes scarlet fever in some cases and puerperal fever in others. *M. catarrhalis* produces symptoms of many diseases—common cold, influenza, scarlet fever, diphtheria, typhoid fever, cerebro-spinal fever.

(4) In different epidemics different types of disease presented—*B. influenzae* causes epidemics simulating coryza, rheumatic fever, typhoid fever, cerebrospinal fever.

(5) Same train of symptoms follows infection by different organisms—typical rabies due to *B. diph.*—typical scarlet fever, cerebrospinal fever and influenza due to *M. catarrhalis*—typical cerebrospinal fever due to *B. typhosus* and to Klebs-Loeffler bacillus—symptoms resembling diphtheria due to pneumococcus—typical typhoid fever due to *B. coli.*

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CHAPTER X

SUMMARY

All characters of bacteria show variation—"spontaneous" or "impressed" —modifying influences already discussed (Chap. II). Variation may be apparent only. Apparently spontaneous variation may be due to unrecognised influences. Variation itself may be specific (morphology of *B. diphtheriae* and *S. scarlatinae*). No one character specific—variation need not imply loss of specific character (morphology of *B. coli*). Analogy of regiment of soldiers and crowd of pitmen. Variation may be specific because it indicates racial character. Many variations represent past stages in evolution (Morphology, Chap. IV)—others represent new steps in evolution (Fermenting Power and Virulence, Chaps. V and VI).

TRANSMUTATION DIFFERS FROM VARIATION IN DEGREE ONLY—different species derived from a common stock—differentiation more advanced in some than others—reversion therefore differently interpreted—necessity of regarding characters as a whole and their stability—danger of relying upon one character alone already shown (Chap. IV–VII). Analogy of human race groups. Variation may indicate recent environment—and so reveal source of particular strain (streptococcus from milk—general coli infection from biliary passages).

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production by *B. ruber*—mycelial development of *B. diph.*). Tendency to revert does not imply loss of specific character. Variation differs from transmutation in degree alone.

TRANSMUTATION DIFFERS FROM EVOLUTION IN DEGREE ALONE. Analogy of different branches of family. Possibility of transmutation. Saprophytic and parasitic pneumococcus. Other examples already discussed (Chap. VIII). Experiments suggesting transmutation already discussed (Chap. IX). 1st series, strains not guaranteed pure, results explained by variation. 2nd series, results explained by variation, secondary invasion not excluded. Transmutation improbable. Enzyme theory of disease. **(140—152)**

CHAPTER XI

THE ENZYME THEORY OF DISEASE

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INTRODUCTION

THE mediaeval alchemists conceived the idea of the transmutation of metals and dreamt of changing the baser metals into gold. The task which baffled them the scientists of our own generation seem destined to achieve. The transmutation of bacteria is a problem of more recent date but it bears a certain resemblance. If silver and gold are the currency of wealth by means of which it changes hands, bacteria represent the currency of disease by means of which this also is passed from one person to another. The resemblance, however, goes much deeper than this, for just as the metals have hitherto been regarded as "elements" of matter so the functions of the unicellular organism have been thought to represent the "elements" of life. The physicist has learnt that the so-called "elements" of matter are themselves composed of infinitely small particles or "ions"; the pathologist is learning that the functions of bacteria in many cases result from the activity of ultra-microscopic bodies, of the nature of "enzymes." The occurrence of transmutation in the case of bacteria would prove as revolutionary in our conception of disease as its occurrence in the case of certain rare metals is already proving in our conception of matter.

The idea of the permanence of characters in the animal world is at least as old as the question "Can the Ethiopian change his skin or the leopard his spots?" but it is only in recent times that the fixity of animal species has been scientifically demonstrated.

Amongst the less highly organised structures of plant life variation is of more frequent occurrence and, though it is not possible to "gather figs from thistles," it is generally acknowledged that "species" in the case of plants are less rigidly defined than in the animal world.

In the realm of bacteriology still simpler forms are met with in which are recognised the beginnings of both animal

and vegetable life, and amongst these variation is of still greater frequency. This fact, confirmed by personal observation and by a perusal of the literature of the subject, suggested to the writer the question whether actual transmutation of species might not occur amongst bacteria, and it was in the hope of answering this question that the investigation here recorded was undertaken.

An endeavour was made in the first instance to collect the published records of all experiments in which transmutation was alleged to have occurred. These were found to be few in number. In the second place, a series of experiments was carried out by the writer with the object of disproving the contention put forward in one of these cases. Thirdly, with a view to criticising the claim made in the remaining cases, and in the hope that it might throw some light on the problem of transmutation as a whole, a study of the subject of variation amongst bacteria was undertaken. The material on which it is based has been collected from the scattered literature of the subject. With a few exceptions, only papers written in English have been consulted.

In Chapter I the scope of the enquiry is outlined. In Chapter II the conditions which modify the characters of bacteria are enumerated and in Chapter III the value of the evidence adduced in proof of such modification having occurred is considered. Examples of variation are then studied in detail and their significance is discussed, reference being made more particularly to morphological characters, fermenting properties, virulence, and pathogenesis (Chapters IV—VII). In Chapter VIII the possibility of transmutation occurring in the animal body is considered. In Chapter IX instances of supposed transmutation are examined. In Chapter X the subject is reviewed at length and the results of the investigation summarised. In Chapter XI the Enzyme theory of disease is discussed, together with its bearing upon the subject of transmutation. In Chapter XII the author's conclusions are briefly stated. References are given in the Appendix.

CHAPTER I

THE SCOPE OF THE ENQUIRY

Definition of Terms

The phrase "transmutation of bacteria" is not synonymous with "evolution of bacteria." "Evolution" is the gradual development of new species and tends towards further differentiation. "Transmutation" is the changing of members of one recognised species into those of another and, if proved, would tend towards unification by undermining existing barriers.

There is no reason to doubt and abundant evidence to support the opinion that in this field of life, as in others, the forces of natural selection and the survival of the fittest have been at work and have resulted, in the course of ages, in the evolution and differentiation of the various types of bacteria which we recognise and distinguish today.

Andrewes, in the Horace Dobell Lecture for 1906, "traced the evolution of streptococci from the condition of harmless mineral-feeders, through that of saprophytism in the alimentary canal, to the development of weak powers of parasitism which have culminated, in certain instances, in the fully developed property of aggressive parasitism seen in the streptococcus pyogenes."

He showed how, at different stages, natural selection and the survival of those best adapted to the environment in which they found themselves, resulted in the permanent acquisition of new characters, such as the ability, when they had once entered upon a saprophytic career in the alimentary canal, to flourish most vigorously at the body temperature of their host and to utilise the foodstuffs available in their new habitat; to resist desiccation during the intervals between their discharge from one host and their reception by another; and later still to support themselves in the actual living tissues of the host

and to defend their position there by the manufacture of haemolysins and toxins.

This process of evolution is, no doubt, going on continually in bacteria as in higher forms of life. It is rendered possible in both cases by the occurrence of *natural variation*. This variation in bacteria is of two kinds, namely, *spontaneous* or intrinsic variation between the individuals of a pure culture—that is to say, bacteria derived from a single organism,—and *impressed* variation, the effect of special environmental conditions upon a succession of bacterial generations, due either to the direct reaction of the bacterial protoplasm to the environment, or to selection acting upon slight spontaneous variations and producing a cumulative effect.

It is reasonable to expect that amongst the bacteria natural variation would occur with greater frequency than amongst higher forms of life for, being unicellular organisms, changes in their environment can operate directly upon the germ plasm. Moreover the common method by which bacteria multiply, namely the division of the parent cell into two daughter cells, ensures the ready transmission of any acquired character from parent to offspring. The variation in character may be said to be retained by the daughter cells rather than transmitted to them. Such retention of parental characters by the daughter cells is not, however, invariable. For example, McDonald (1908) has published photographs of a young culture of the meningococcus showing diplococci in which one member is stained while the other is not. Thirdly, such variations would be more readily noted in their case since as many as 30 or 40 successive generations may be observed in the course of 24 hours. In the case of some bacteria division may occur as frequently as once every 17 minutes (Barber, 1908). Yet a fourth factor might be mentioned, namely the ease with which the environment of any strain of organisms can be modified in any direction and to any extent.

As a matter of fact examples of such variation, as we shall show, are innumerable, no matter what particular property or character of bacteria we investigate. Differences occur in size and shape, in staining properties, in power of growth on various

media, in viability, in virulence, in the power to ferment sugars, and so on.

The environment in which bacteria grow and multiply tends, in the course of time, to "fix" some of these variations by offering to the possessors of them a better chance of survival or perpetuation, so that they ultimately become characteristic of a new species. This is evolution through *natural selection*.

By a similar process of *artificial selection*, as will be shown, we can encourage variation in almost any direction we choose. "Within certain limits the simple forms of life are able to adapt themselves to their surroundings and the adaptation cannot be ascribed to chance for, with a given environment, the one particular alteration in properties surely results." (Adami, 1910.) If we so vary the characters of a member of one species that it comes ultimately to possess all the characters of a member of another species, that is "transmutation." The question is, how far can we go in this direction, and to what extent are the recognised species of bacteria really fixed in their characters?

The Meaning of Species.

The objection may be raised at this point, that the phrase "transmutation of species" involves a contradiction in terms, since the very definition of "species" excludes the possibility of transmutation. This leads us to a further question, namely, what do we mean by the word "species" as applied to bacteria?—in other words, what determines our present classification?

The distinction between different species of bacteria and their recognition depends upon the observation of their characters—morphological, biological, chemical, physiological and pathological. Briefly enumerated these are as follows:—the naked eye appearance of colonies and of a stab culture: microscopic appearances, size, shape, motility, adhesiveness: method of generation and life history, involution forms: power to produce pigment: staining properties: cultural characters, power of growing on different media, in the presence or absence of oxygen, and under different conditions of temperature and

moisture, with or without production of gas or odour; power to liquefy gelatin, to reduce neutral red, to clot milk, to ferment various carbohydrate substances: power to form agglutinins and susceptibility to agglutination: viability under different conditions: virulence or the nature of the toxins they produce: pathogenicity or the nature of the lesions they cause and the kind of animal susceptible to their invasion.

It is seen from this list that the characteristic qualities of bacteria are very numerous and it would be thought that their classification would on this account be very thorough and complete. But, as will be shown in the course of this enquiry, every one of these characters is liable to variation and the occurrence of these variations renders the task of classification very difficult and in many cases uncertain.

If certain characteristics were invariable, even though others varied, a definite criterion would be afforded, but where all alike are subject to modification the division into species is necessarily an arbitrary one. Amongst the higher animals, where sexual production prevails, mutual fertility or sterility offers a guide in determining the limits of species. Here no hard and fast line can be drawn. Nevertheless we see exhibited amongst bacteria, in the words of De Bary, "the same periodically repeated course of development within certain empirically determined limits of variation," which is considered to justify the recognition of a species.

Many species of bacteria do show characters apparently quite fixed and rigid. The anthrax bacillus and the tetanus bacillus are quite as good species in the natural history sense as any that can be found amongst flowering plants. But the classification of others is still a matter of dispute. This can be illustrated by reference to the streptococci.

The Classification of the Streptococci.

Marmorek held the opinion that the human streptococci constituted one species. "He based his chief argument on the observation that bouillon in which one sort of streptococcus had grown would not serve afterwards as a culture medium for any other streptococcus, and that the same haemolytic

power was possessed by them all." (Andrewes and Horder, 1906.)

In 1891 von Lingelsheim (*ibid.*) proposed a division into two groups according to the length of chains formed: "streptococcus brevis" and "streptococcus longus." Andrewes and Horder (1906) with a view to further classification on the same lines suggested the adoption of the terms brevissimus, brevis, medius, longus, longissimus, conglomeratus. The quality of cohesiveness by itself was, however, considered too trivial a character to base a fundamental classification upon.

The power of retaining stains was found to offer no means of differentiation, since all stained well.

Minute differences in their mode of growth on different media were found to be too inconstant to be of any value, though Schottmüller (quoted by Muir and Ritchie) attempted to classify the streptococci according to the appearance of colonies.

Classification according to pathogenicity and virulence appeared to have the advantage of being practical and significant from a clinical standpoint. Virulence, however, was likewise found to be an inconstant character, being lost and regained with great readiness by these organisms. It was lost after a few days on certain culture media. On the other hand, after a few "passages" through a susceptible animal, a streptococcus of feeble virulence might become intensely pathogenic. Clinical experience confirms this variability in virulence. "One and the same strain of streptococcus may at different stages in its career produce now a rapidly fatal septicaemia, now a spreading erysipelas, now a localised suppuration and now no effect at all" (Andrewes and Horder, 1906), so that the degree of virulence was an uncertain aid to classification. The streptococcus erysipelatis, for instance, is no longer considered on account of its virulence to be a distinct species from the streptococcus pyogenes.

Agglutination tests have not been found to be sufficiently specific.

Marmorek's contention, therefore, for the unity of species of the human streptococci continued to hold the field successfully until the introduction of Gordon's tests.

Gordon (1903–4) isolated from human saliva 300 strains of streptococci. He tested separately the power of these different strains to ferment various substances, consisting of 14 carbohydrates, 13 glucosides, and 6 polyatomic alcohols. Many of these test substances proved of no differential value as regards streptococci, either because they were uniformly attacked by all or because no streptococci could ferment them, but he was led to select a series of seven substances—namely saccharose, raffinose, inulin, salicin, coniferin and mannite—as being of special value as tests for streptococci, and to these he added two further tests—the clotting of milk and the reduction of neutral red under anaerobic conditions. By such means he was enabled to distinguish 48 chemical varieties.

Houston (1903–4) applied the same tests (with the omission of one—the action on coniferin) to 300 strains of streptococci derived from human faeces and was able to distinguish 40 chemical varieties amongst them.

Gordon demonstrated that these chemically different strains were remarkably constant in their reactions and this was confirmed later by the work of Andrewes and Horder (1906), who tested his strains and, in addition, some 200 new strains derived from foci of disease in human beings. "Gordon himself was careful to abstain from claiming specific value for his different chemical types and he did not venture to propose any reasoned scheme of scientific classification based upon his tests." Andrewes and Horder attempted to do this. They collected from various sources particulars of the behaviour of over 1200 different strains of streptococci when subjected to Gordon's tests. As a result they found that these 1200 strains fell into some half a dozen main groups. By adding one further test—the power of growth in gelatin at 20° C.—and by taking into consideration also the morphological characters and pathogenesis, they were able to define five varieties of streptococci which they regarded as of "approximately specific value" though connected by a multiplicity of intermediate varieties. They named these *S. anginosa*, *S. salivarius*, *S. faecalis*, *S. pyogenes* and *S. pneumococcus*.

Though confirming, on the whole, the stability of the

reactions constituting the tests, these observers noticed that variation in virulence was sometimes accompanied by changes in chemical behaviour. They also acknowledged that slight differences in the composition of the media might possibly affect the series of reactions to some slight extent.

Subsequently Ainley Walker (1911) offered evidence to show that greater differences in the media do actually affect the reactions to a remarkable extent, so much so as, in his opinion, to invalidate any claim that they should be regarded as specific, and he fell back on the position held by Marmorek.

Still later Jensen and Holth, after a prolonged investigation, came to the opposite conclusion, that is to say in favour of the stability of the differences brought out by Gordon's tests, but they also showed that these differences were in no way closely related to virulence or pathogenic action so that the method of classification founded on them was imperfect.

We have thus demonstrated that the term "species" when applied to bacteria must be interpreted much more loosely than in the case of plants or the higher animals and the phrase "transmutation of species" is thereby absolved of the accusation of being self-contradictory.

The aim of this paper is to show how far transmutation does occur, and what is its significance.

It will be obvious, from what has already been said, that in considering the evidence of transmutation it will be necessary to consider also the evidence of variation. The difference between the two is one of degree only. A member of one "species" of bacteria is distinguished from a member of another "species" by its morphological and other characters. If these characters become altered, within certain limits, the process may be regarded simply as variation; if outside these limits it must be regarded as transmutation.

We have first of all to consider then, what are the possibilities in the direction of such alteration in character.

A Consideration of the Possibilities.

The various possibilities to be considered are five in number. It will be seen that the first three are instances of variation and the remaining two, instances of transmutation.

1. *Simple variation.* Modifications may occur in the characters of an organism, involving either the loss of some feature previously regarded as characteristic or the acquisition of some other feature not hitherto considered to be so,—such modifications not being so numerous, however, or so fundamental, as to lead to any doubt as to the proper identification of the organism. For example, Twort (1907) succeeded, in the course of two years, in training a strain of *B. typhosus* to ferment lactose, and both Twort (1907) and Penfold (1910 A) produced pure strains capable of fermenting dulcite, which the usual variety of typhoid bacillus is practically unable to do. These new strains retained qualitatively all the other properties of the *B. typhosus* unchanged. Similarly Miss Peckham (1897) induced indol formation in numerous strains of *B. typhosus.*

2. *Variations in different directions associated.* The acquirement of some fresh character may be associated with the loss simultaneously of some other character previously possessed or, with the acquisition of a second new character, and in some cases this association may prove to be invariable under the same modifying conditions (*vide* p. 146). For example, Eyre and Washbourn (1899) observed that a non-virulent strain of the pneumococcus growing readily at 20° C. could by "passage" be converted into a highly virulent strain which was then unable to grow at a temperature below 37° C. The reverse change showed the same relation between the virulence of the organism and the temperature at which it would grow.

Jenner (1898) was able to revert *B. coli capsulatus* to an unencapsulated form by cultural methods and found that the new variety had lost the power to coagulate milk, and instead of being highly pathogenic to white mice had become much less so or even non-pathogenic.

The acquirement of power to ferment a certain carbohydrate may coincide with the loss of fermenting power in other directions.

Penfold (1910–11) has shown that the development of new fermenting powers on the part of *B. typhosus* towards lactose and dulcite is frequently associated with the formation of papillae on its colonies. Diminished gas-production in glucose media on the part of certain coliform organisms (*B. Grünthal*, etc.) was likewise associated with papillae formation.

The same observer found that colonies of *B. typhosus* which had lost the property of fermenting glycerine showed impaired agglutinability also, though typical fermenting colonies on the same plate were normal as regards agglutination.

Adami, Abbott and Nicholson (1899) found that the assumption of coccic and diplococcic forms by *B. coli* in the organs of healthy animals was associated with a loss of power to ferment carbohydrates and to produce indol.

Gordon (1900–1) observed that the tendency of the streptococcus of scarlet fever to assume a bacillary form was abolished by "passage" and at the same time its virulence was increased.

Rosenow (1914) obtained a strain of streptococci from the throat in a case of scarlet fever, which yielded on blood agar two distinct kinds of colonies. These displayed marked differences in their fermenting power and also in their pathogenicity.

Many other instances might be given.

3. *The development of intermediate forms*, i.e. the possible derivation from one or other of two known species of forms intermediate between them in their characters. For example, W. J. Wilson obtained from the urine of a supposed typhoid carrier (1910), and also from the urine in certain cases of cystitis and pyelitis (1908), coliform organisms intermediate in their characters between *B. typhosus* and *B. coli communis* and derived presumably from *B. typhosus* in one case and from *B. coli* in the others. Many other observers have described organisms resembling both *B. typhosus* and *B. coli communis* in their characters. Klotz (1906) has described such an organism, isolated from water, and called by him *Bacillus perturbans*. Mcnaught (1905) described two varieties,

also derived from water, under the term *Bacillus typhosus simulans.*

One organism, for example, described by Wilson (1910), resembled *B. typhosus* in forming acid without gas in glucose and in failing to ferment lactose at 37° C.; but it failed to agglutinate with typhoid serum, and it resembled *B. coli* in producing acid and much gas from mannite and in fermenting lactose at 22° C.

The *Bacillus perturbans* of Klotz was agglutinated by high dilutions of typhoid serum (1–1550 in 15 minutes) and it produced slight acidity in milk without coagulation; but it differed from *B. typhosus* in fermenting both lactose and saccharose, in giving the neutral red reaction and forming indol, and in other ways.

Major Horrocks obtained from the urine of a patient convalescent from typhoid fever a typical strain of *B. typhosus* which however on subculture gave rise to an organism intermediate in its characters between *B. typhosus* and *B. coli* (*vide* p. 118).

4. *Slight changes in closely allied organisms,* i.e. the possibility in the case of closely allied organisms of a modification in the few distinguishing features they possess, so that they may appear to change, the one into the other. For example Schmitt (1911) concluded from his experiments that paratyphoid bacilli of the 'Flügge' type and of the 'Gaertner' type, generally regarded as distinct species, could be transformed from one into the other in the animal body.

5. *A complete change in characters,* i.e. the possibility of the occurrence, more or less suddenly, of a complete change simultaneously of all the characters of an organism, or at least of all the fundamental ones by which it was distinguished. For example, Major Horrocks (1911) concluded that he had been able gradually to modify a strain of *B. typhosus,* by changes in its environment, to such an extent that it assumed eventually the characteristics of a Gram-positive coccus having the cultural characters of *Streptococcus faecalis.*

Before these several possibilities are studied more fully, the conditions which modify the characters of bacteria will be mentioned and examples given under each head.

CHAPTER II

CONDITIONS MODIFYING THE CHARACTERS OF BACTERIA

THE factors which appear to influence the growth and development of bacteria and to produce modification in their characters are many in number and diverse in nature, and it is often impossible to state with certainty which of these various factors is the one primarily responsible for the modification observed in a particular case.

1. Many variations appear to be *spontaneous*, not due, that is to say, to any external agency, but the result of developmental or atavistic tendencies inherent in the organism itself. We know as little of the nature of these tendencies to variation as we know of the nature of those which control normal development and, in the vast majority of cases, prevent variation occurring.

Some spontaneous variations are examples of "*pleomorphism*" and represent stages in the life history of the individual organism or of the race. Others cannot be explained in this way. For example, one component of a diplococcus may retain a stain while its fellow fails to do so. In such a case faulty technique cannot be held responsible, nor can the variation be attributed to differences in environment. Moreover in the case of the meningococcus it has been found to persist after animal passage (McDonald, 1908). The variation would appear to date from the cell division which constitutes the "birth" of the organism. Denny (1903) observed the same inequality in staining properties in different segments of a segmented form of *B. Xerosis*.

Many other examples of spontaneous variation will be found in later pages.

2. Differences in characters are sometimes associated with differences in *geographical distribution*. Thus Schultz (1909)

found that in Cleveland U.S.A., during the 12 months covered by the investigation, "barred" forms of the diphtheria bacillus had almost disappeared; during the same period, in Boston and Providence, another observer noted that "barred" forms were unusually common while "granular" forms were very rarely met with.

3. Many organisms after *prolonged cultivation* on artificial media display variation in character.

In some of these cases the length of the period of cultivation is not the cause of the modification, it merely *extends the survey* over a large number of generations and so enables the observer to detect variations spontaneously occurring.

In other cases the length of time *permits "natural selection" to play its part* and produce modifications which, in a shorter interval, would not have advanced far enough to be apparent.

In other cases, again, the prolonged exclusion from animal tissues does lead directly to a modification in character which is proportionate, as regards its extent and its permanence, to the duration of such exile, but disappears when the organism is again "passed" through the body of an animal. This is true more particularly of the property of virulence (*q. v.*).

As an example of the influence exerted by prolonged cultivation in modifying the character of an organism may be cited the statement of Mohler and Washburn (1906) that a strain of bovine tubercle bacilli after cultivation for 11 years was found to have become modified in morphological and cultural characters to the human type.

Lentz (quoted by Bahr, 1912) found that a "Flexner" type of *B. dysenteriae* after 9 years' laboratory cultivation completely lost the power to ferment maltose. Arkwright (1909) mentions a strain of the meningococcus which when first isolated did not ferment glucose but after ten months' artificial cultivation developed power to do so. Rettger and Sherrick (1911) describe the gradual loss of power to produce pigment on the part of an old stock culture of *B. pyocyaneus* after 5 years' artificial growth.

Prolonged cultivation in a medium containing a particular carbohydrate may develop in a strain of bacteria the ability

to ferment that carbohydrate. *B. typhosus* for example after two years' growth in a medium containing lactose acquires the power to ferment this sugar (*vide* p. 58).

4. In other cases again the length of the period of cultivation is of less importance than the *conditions under which such cultivation takes place.*

(*a*) *Conditions which lower the vitality* of a strain may modify its characters. Such conditions include starvation (for example, growth in pure water), acidity of the medium, want of oxygen, the presence of antiseptics, exposure to sunlight, high or low temperatures, symbiosis, etc.

One example will suffice. A strain of *B. ruber* of Kiel if heated to a temperature just below that known to kill the organism loses its power to produce pigment (Adami, 1892).

(*b*) The *crowding together* of the organisms on the surface of the medium may lead to a diminution in pigment production in the staphylococcus aureus (Andrewes and Gordon, 1905–6) and an earlier appearance of granular staining forms of the diphtheria bacillus (Denny, 1903).

(*c*) The *temperature* at which organisms grow is responsible for certain variations. Laurent (1890) found that a selected strain of *B. ruber* which had grown for 12 months at a temperature of 25°—35° C. without exhibiting a trace of colouration, yielded its characteristic pigment when the temperature was lowered to 18° C. An apparent staphylococcus "albus" growing at 37° C. may become a vivid "aureus" at 22° C. (Andrewes and Gordon, 1905–6).

The virulence of *B. anthracis* is greatly modified by growth at 43° C. and that of *B. diphtheriae* may be destroyed by subjection to a similar temperature (Hewlett and Knight, 1897).

Wilson (1910) describes an atypical *B. typhosus* which fermented lactose at a temperature of 22° C. but failed to do so at 37° C. Coplans (1909) found that dulcite was more quickly fermented by certain colon bacilli at 20° C. than at 37° C.

Rodet (quoted and confirmed by Adami, Abbott and Nicholson, 1899) found that at a temperature of 45° C. *B. coli* developed in a few hours into long filaments. The same

agency will abolish the power of some bacteria—such as *B. anthracis*—to form spores, and may modify the character of the colonies it forms (Bainbridge, 1903).

Bacteria of the paratyphoid group agglutinate much less readily after being heated (Sobernheim and Seligmann, 1910).

(*d*) Differences in *atmospheric pressure* may modify the activity of certain organisms.

B. coli yields formic acid and gas from glucose at ordinary atmospheric pressure. If the pressure is raised the yield of gas diminishes but the yield of formic acid increases (Harden, 1901).

A great pressure of carbon dioxide is said to deprive *B. anthracis* of its power to form spores though it has no effect on the vitality of the organism (Muir and Ritchie).

Certain organisms which do not readily lose their virulence on artificial media do so rapidly if grown in an atmosphere of compressed air (*ibid.*).

(*e*) The presence or absence of *oxygen* is another factor of importance. Strains of *B. typhoid* and *B. coli* growing in water maintain their viability better if plentifully supplied with oxygen (Whipple and Mayer, 1906).

In the absence of free oxygen *B. pyocyaneus* ceases to produce pigment (Adami, 1892) though the *spirillum rubrum* produces it more plentifully (Muir and Ritchie).

Torrey (1905) observed that by alternate aerobic and anaerobic culture a certain type of dysentery bacillus had its power to ferment maltose greatly augmented.

Andrewes and Horder (1906) found that a certain streptococcus which refused to ferment lactose, under ordinary conditions of cultivation, did so readily when deprived of oxygen.

Kruse (quoted by Glenn, 1911) found that a staphylococcus which, similarly, refused to liquefy gelatin did so at once under the same altered conditions.

Anthrax bacilli in the absence of oxygen may develop torula zoogleic forms (Wood, 1889). Noguchi (1910) discovered that *B. bifidus communis* only exhibited the bifurcating phase under anaerobic conditions and in the absence of oxygen became less pathogenic.

It is well known that *B. diphtheriae* produces toxins more plentifully in a free supply of air (Clark, 1910).

The bacillus of malignant oedema is said to lose virulence when grown aerobically (Harass, 1906) and that of cholera to gain virulence when grown anaerobically (Hueppe, quoted by Adami, 1892).

Foa (1890) describes how a strain of the pneumococcus could by anaerobic growth be deprived of its property of causing a characteristic inflammatory oedema of the skin when injected into an animal.

Wood (1889) attributed the diminished infectivity of virulent organisms discharged from the bowel in many diseases to the fact that in the bowel they are practically deprived of oxygen.

(*f*) *Bright sunlight* destroys the virulence of some pathogenic organisms (Marshall Ward and Blackman, 1910) and leads to the loss of pigmentation in others (Laurent, 1890). The bacterium mycoides, on the other hand, will only produce its red pigment in the dark (Scholl, quoted by Wood, 1889).

5. Exposure to the *ultra violet rays* has recently been shown by Madame Henri (1914) to effect a startling change in both the morphology and the pathogenicity of *B. anthracis*. Cocci and filamentous forms were produced which differed from the original bacilli in their power of retaining stains, of forming spores, of liquefying gelatine and coagulating milk, and which gave rise, on injection into an animal, to symptoms quite unlike those produced by normal anthrax bacilli. The new forms did not revert after daily subculture for over two months.

6. *Electrolysis.* Electrolysis may produce changes in the morphology and staining properties of bacteria. Russ has observed the production of elongated forms of *B. coli*, with altered reaction to Gram's stain, in urine (within the human bladder and outside the body) as a result of the passage of a galvanic current of $\frac{1}{15}$th m.a. strength for one hour. The modification persisted for many months.

7. The *age of the culture* is of importance in the case of many pleomorphic organisms. For example, *B. megatherium*

and *B. subtilis* pass in a few hours from a bacillary motile stage with cilia, to one of filamentous growth preceded by the casting off of cilia (Marshall Ward and Blackman, 1910).

This factor influences the characters not only of bacteria known to be "pleomorphic" but of others also. Young tubercle bacilli are said not to be "acid-fast" (Hamer, 1900).

Young cultures of *B. diphtheriae* more often show branched and clubbed forms (Kanthack and Andrewes, 1905) and solid staining bacilli (Denny, 1903) than do older cultures; at the same time they are unable to ferment glycerine and lactose though older cultures usually ferment both (Muir and Ritchie).

A young culture of *B. coli* does not yield indol (MacConkey, 1909). Wood (1889) found that an old culture of cholera failed to liquefy gelatin but did so readily after subculturing, and that a young culture of the same organism was much more susceptible to the action of antiseptics than a younger one.

Old cultures of pigment forming bacteria are often colourless (Adami, 1892).

Arkwright (1909) found bacillary forms of the meningococcus in old cultures. The tubercle bacillus also in old cultures displays elongated and even branched forms.

8. *The character of the culture medium* employed may influence bacteria in many ways.

(*a*) The *age of the medium. S. pyogenes* normally does not ferment saccharose, raffinose or salicin, but if old media be used this organism will ferment all three substances (S. Martin, 1908–9). On the other hand *B. diphtheriae*, which in fresh beef serum gives its characteristic "sugar" reactions, fails to do so if this medium is old (Fisher, 1909).

(*b*) Changes in the *reaction of the medium* employed is in many cases accompanied by changes in the morphological characters of organisms growing in it, bacilli giving place to cocci and diplococci, and *vice versa*, and pigment formation being modified or lost (Adami, 1892). The reaction also affects the vitality of many bacteria (Wood, 1889), and their virulence (Peckham, 1897).

(*c*) The *nature of the medium* is important. Individual morphology, the appearance of colonies, fermenting power,

indol formation, pigment production, and virulence, all vary with the kind of medium used.

Gordon (1900–1) states that the streptococcus of scarlatina may form, on serum, rods which closely resemble *B. diphtheriae* but in a liquid medium it grows in a typical streptococcal form.

B. diphtheriae does not form toxins readily if there is much carbohydrate present in the medium (Fisher, 1909).

Many media contain substances derived from the living body such as serum, or blood, and to this extent are "natural" rather than "artificial" media and the alteration in the character of organisms growing in them, particularly as regards virulence, is possibly to be attributed to this factor.

Penfold (1914) mentions the fact that vaccination with a plague strain grown on agar will protect rats against itself but not against the same strain grown on serum.

If the ordinary artificial media are replaced by the *natural secretions of the body* the modifications in character on the part of the organisms growing in them may be even more marked.

Rosenow (1912–13) found that a streptococcus which presented certain morphological and cultural characters on ordinary media, underwent a profound modification in respect to both as a result of growth in unheated *milk*.

Horrocks (1911) found that a strain of *B. typhosus*, obtained from the urine of a "carrier," lost virulence on ordinary media (broth, and agar) in a few days but maintained it for over a year in *urine*.

Both in milk and in urine *B. coli* may form a dense network of branching filaments (Revis, 1908, Wilson, 1908) and give atypical fermenting reactions (*ibid.*).

In the presence of *saliva B. coli* yields leptothrix forms (Adami, Abbott and Nicholson, 1899), while *S. mastitidis* is deprived of virulence (Savage, 1908–9).

Diplococcic forms of *B. coli* occur in *bile* (Adami, Abbott and Nicholson), while in *ascitic fluid* the same organism undergoes profound changes in respect both to its morphology and its fermenting power (*ibid.*).

Pathological exudations influence the characters of bacteria growing in them to an even greater degree than the natural secretions. This is particularly true of virulence (*vide* p. 77).

Harris (1901) examined 15 strains of *B. coli* from "natural" sources—such as sewage, water, milk, shellfish—and also 11 strains from "diseased" sources, that is to say from inflammatory exudations. Of the former, only two were virulent; of the latter only one was non-virulent.

Growth in water also influences bacteria. *B. coli* in river water, where they are practically deprived of proteid food, appear to lose their power of producing indol (Peckham, 1897). The same organism isolated from drinking water was found by Savage (1904) to form less typical colonies than when isolated from sewage or faeces, while Jenner (1898) describes its morphological appearances as being different, bacilli isolated from water being less thick and opaque—a distinction which disappeared when the strain was grown in milk.

(*d*) The *addition of various chemical substances*, such as antiseptics, to the media used for cultivation profoundly modifies the development of bacteria. In the presence of carbolic acid typhoid bacilli assume the form of non-motile cocci and diplococci (Adami, 1892), the bacillus anthrax loses virulence and also the power to form spores (Roux, 1890), many bacteria no longer liquefy gelatin (Wood, 1889), while others lose their power to ferment carbohydrates (Penfold, 1911 B). Under the influence of antiseptics *B. prodigiosus* forms spirillae and ceases to produce pigment (Wasserzug, 1888). The bacillus of blue pus—normally a small short bacillus—yields, on the addition of a trace of boric acid to the medium, S-shaped forms and close spirals, and on the addition of potassium bichromate, long undulating filaments (*ibid.*). The presence of sodium benzoate inhibits gas production in the case of *B. coli* (Herter, 1909). The addition of glycerine prevents the liquefaction of gelatin by bacteria (Adami, 1892) and also inhibits the formation of indol (Wood, 1889). A virulent *B. diphtheriae* is promptly attenuated by the addition of iodine trichloride to the medium (Mohler and

Washburn, 1906) and a non-virulent bacillus of Blackleg rendered virulent by the addition of lactic acid (*ibid.*). Increased resistance to antiseptics may be developed by prolonged exposure to their action (Rettger and Sherrick, 1911: Penfold, 1911 C).

Many other examples are given in later pages.

9. Unusual fermenting properties on the part of bacteria are frequently acquired after *prolonged growth on special sugar or peptone containing media.* The length of time required varies in different cases, within wide limits. For example, *B. typhosus* can be "trained" to ferment dulcite in less than two weeks (Penfold, 1910 A) but cannot be trained to ferment lactose in less than two years (*ibid.*). The method is not invariably successful, but this may be due to the fact that the trial in many cases is not sufficiently prolonged.

Unusual proteolytic powers may be developed in a strain of organisms by an analogous process. Miss Peckham (1897) induced the power to form indol on the part of many strains of *B. typhosus* by cultivating them in a medium rich in peptone.

10. By *artificial selection*, through a number of generations, it is often possible to develop variation in a particular direction.

Goodman (1908) by this method obtained from a strain of *B. diphtheriae* with moderate power of producing acid in dextrose broth, two strains possessing respectively a greatly augmented and a greatly diminished power of acid production.

Rettger and Sherrick (1911) in the same manner obtained from a slightly pigmented strain of *B. prodigiosus* two strains showing in one case brilliant colouration and in the other complete absence of colour. They also obtained by selection a strain of staphylococcus aureus unusually resistant to the action of corrosive sublimate.

Conn (quoted Glenn, 1911) obtained by the same method strains of a micrococcus with high and low powers of liquefying gelatin.

This method, however, may prove unsuccessful. Rettger and Sherrick failed to modify pigment production in *B. ruber balticus* and they quote Buchanan and Traux as having been

unable to establish high and low acid producing races of streptococcus lacticus. Glenn (1911) failed to produce high and low acid producing strains of *B. proteus*. Other observers have failed in attempts by selection to develop a particular morphological type of the diphtheria bacillus (Clark, 1910) and to modify the agglutination reactions of *B. typhosus* (Moon, 1911).

11. *Symbiosis* is known to influence the behaviour of bacteria and has in many cases a marked effect on the character of one or other of the organisms growing together. The phenomenon of symbiosis is a familiar one in vegetable life. The individual struggle for existence is observed to give place occasionally to a permanent partnership between two organisms for their mutual benefit. An example of such cooperation is furnished by the Lichens each of which is a dual organism composed of a fungus and an Alga. Vegetable life as a whole is dependent upon the activity of nitrifying organisms in the soil and in some cases a definite alliance is formed between the two parties, as in the case of the Leguminosae and the nitrifying bacteria which take up their residence in the root nodules of these plants.

All forms of mutual parasitism are in reality examples of symbiosis. One species of bacteria may, however, be dependent for its growth upon another species without necessarily being parasitic. Thus Pasteur advanced the theory that aerobic bacteria by exhausting the supply of oxygen gave anaerobic bacteria a chance of growing.

Allen (1910) found that a strain of *B. influenzae*, which could not be grown on ordinary media, grew luxuriantly on sterilised media in which the staphylococcus albus had previously grown. Neisser was able to cultivate the same bacillus on plain agar for several generations by growing the Xerosis bacillus with it, though a dead culture of the latter had not the same favouring effect (Muir and Ritchie).

In other cases the growth of one species is inimical to the growth of another. *B. typhosus* will not grow in a filtered broth culture of staphylococcus albus, nor in that of many other organisms (Freudenriech, 1888). The meningococcus is

inimical to the growth of the Klebs-Loeffler bacillus (Smirnow, 1908).

Prescott and Baker (1904) describe a similar antagonism between streptococci and *B. coli* and they attribute the extinction of the latter when the two are grown together to the greater sensitiveness of *B. coli* to the lactic acid produced by both combatants.

Klein (1903–4) found that a strain of *B. typhosus* was killed by *B. coli* in the peritoneal cavity and Horrocks (1911) found that the same thing happened in water which contained both organisms. Jordan, Russell and Zeit (1904) observed that *B. typhosus* quickly died out in polluted water.

Symbiosis is observed to influence not only the viability of bacteria but their virulence, their morphology, their fermenting powers, and other characters. The presence of the streptococcus is said to be inimical to the growth of the diphtheria bacillus (Smirnow, 1908) but it increases the virulence of the latter during "passage" (Muir and Ritchie, 1910) while on artificial media it induces changes in the morphology of the bacillus, "granular" forms appearing earlier than in a pure culture (Denny, 1903). Smirnow (1908) observed that the same bacillus grown on agar in the presence of a bacillus isolated from an acute rhinitis, assumed a coccic form—retaining, however, its virulence unimpaired. The meningococcus produced a similar change.

Lesieur (1901, quoted Clark, 1910) claimed that the pseudo-diphtheria bacillus may assume the morphological characters of the Klebs-Loeffler bacillus as a result of symbiosis with aurococcus aureus.

Horrocks (1911) found that a typical strain of *B. typhosus* lost its power to ferment "sugars" when grown in the presence of a strain of *B. coli* derived from a typhoid carrier (*vide* p. 119).

In some cases bacteria growing together are able to produce results which neither can do alone. For example, neither *B. coli* nor *B. dentrificans* alone can reduce nitrates, but if allowed to act on sodium nitrate together they bring about the escape of free nitrogen (Marshall Ward and Blackman, 1910).

In other cases the prolonged growth of two species together appears to produce no change whatever in either of them. Williams (1902) grew a virulent streptococcus and a virulent diphtheria bacillus together, transplanting every three or four days for 90 such "generations" without influencing the characters of either organism. Horrocks (1911) grew *B. typhosus* and *B. fluorescens non-liquefaciens* together for a period of four months. Examinations made at intervals of one week throughout this period revealed no alteration in the character or agglutination properties of the *B. typhosus.*

The *methods* of studying the effect of symbiosis are various. Simultaneous growth can be studied by "sowing" different species of bacteria together indiscriminately on the surface of the medium; or distinct colonies may be grown on a plate so that at first a considerable space intervenes between colonies of the different species, the interval gradually lessening as the colonies extend until it is finally obliterated; a third method is that of "criss-cross" planting, the effects of symbiosis being seen at the intersection of the lines of growth; or fourthly, one species may be grown on the surface of the medium and another deep to it in the form of buried colonies; or fifthly, a double celluloid sac may be utilised in which the products of bacterial growth can diffuse from one compartment to the other; finally, successive growth offers a further means of investigation, the medium being sterilised after the growth of one species and then planted with the other.

12. The methods of modifying bacteria which remain to be described all involve the agency of the living tissues and may be regarded as forms of *parasitism.*

(*a*) *Transmission through the alimentary canal* is said in some cases to bring about modifications in character. It has been suggested that the bacillus of Aertryck may assume the characters and agglutinative properties of *B. enteritidis Gaertner* after transmission through the intestine of the mouse.

Bahr (1912) found that the fermenting powers of certain strains of dysentery bacilli were modified after they had been passed through the intestine of the fly, although for nine

months previously the strains had repeatedly given normal sugar reactions.

(*b*) *"Passage"* through an animal, or series of animals—by successive injections into the blood, or into the peritoneal cavity, and subsequent re-cultivation from the heart's blood or peritoneal fluid—is known to modify the characters of bacteria in many cases.

Fermenting power, by such means, may be greatly modified (*vide* p. 57). Virulence, again, may in this way be markedly increased towards some animals and diminished towards others (*vide* p. 81).

It is claimed that the pseudo-diphtheria bacillus can be converted into the Klebs-Loeffler bacillus by passage through rabbits (Lesieur, 1901), or through guineapigs (Ohlmacher, 1902).

Adami, Abbott and Nicholson (1899) injected typical *B. coli* into the circulation of a rabbit and obtained diplococcic forms of the organism from the liver after death. They isolated from ascitic fluid in another case similar diplococci which stained irregularly and were non-motile, did not ferment sugars or produce indol, and formed colonies on agar closely resembling those of *S. pyogenes*. By intraperitoneal passages through three guineapigs these organisms were converted into typical *B. coli*.

Schmitt (1911) claims to have so modified a strain of *B. paratyphosus* (Flügge) by passage through a calf that it afterwards gave the agglutinative reactions of *B. enteritidis Gaertner*.

Calves, monkeys, rabbits, guineapigs, rats, mice and birds may all be used for this purpose.

(*c*) A modification of the last-named method consists in growing organisms in a *celloidin sac within the body cavity* of an animal. Martin (1898) increased the virulence of a strain of *B. diphtheriae* by growing it in a celloidin sac in the peritoneal cavity of a rabbit.

(*d*) *Growth in the living tissues* of an animal host is another method of inducing variation. It is only in the animal body that the actinomyces produces its characteristic rays or clubs (Bowlby and Andrewes, 1913).

Ohlmacher (1902) claimed to have changed typical *B. diphtheriae* into Hoffmann's bacillus by 48 hours subcutaneous growth in a rat previously immunised.

The "solid-staining" type of *B. diphtheriae* has been inoculated into a guineapig and the "granular" type has been recovered subsequently from the site of the inoculation (Denny, 1903).

Such a method is not invariably successful. For example, Baldwin (1910) grew the human type of tubercle bacillus in the living tissues of the cow for nineteen months, in the hope of modifying its characters to those of the bovine type, but without success.

(*e*) *During the course of a disease* the organism responsible is not infrequently observed to undergo modification with respect to one or another character. Thus in diphtheria, as convalescence is reached, the "granular" forms of the bacillus give place to "solid-staining" types (Gorham, 1901).

In the chronic stages of cerebrospinal fever the meningococcus isolated from the spinal fluid is found to have lost in some cases its power to ferment dextrose (Connal, 1910). Arkwright (1909) found bacillary forms of the meningococcus in the spinal fluid in several cases of cerebrospinal meningitis.

Adami, Abbott and Nicholson (1899) isolated from the ascitic fluid in cases of cirrhosis, strains of *B. coli* (already described, *vide* p. 25) possessing unusual morphological, cultural and fermenting characters. Similar variants of *B. coli* were obtained from an inflamed gall-bladder.

Foa (1890) injected the pneumococcus into a rabbit and after its death isolated strains from the lung and from the spinal fluid which produced lesions of two distinct types when injected into other rabbits. He proved by experiment that the difference between the two strains was due to differences in the amount of oxygen available for them in the lung and in the spinal canal.

Rosenow (1912–13) describes a certain streptococcus, isolated from cases of epidemic sore throat which exhibited unusual and distinctive morphological and cultural characters. "The strains isolated from the peritoneal exudate and blood

showed them to a greater degree than those isolated earlier in the attack or from the tonsils at the same time. After cultivation on blood-agar it was noticed that the strains from the tonsils soon lost any distinctive peculiarities, whilst those from the exudate retained them longer." He concludes that the unusual character of the organism was directly due to residence in the body fluids and was accentuated as the disease advanced.

Leutscher (1911) found that pneumococci isolated from the lung in acute pneumonia after the crisis were more virulent than those isolated earlier in the illness.

(*f*) In some cases—the so-called "*carriers*"—after all symptoms of a disease have subsided, the particular organism concerned resists all attempts to eradicate it and continues for an indefinite period to grow and multiply at the site of the original lesion. In such cases the organism may become modified in the course of time. This is true more especially of its virulence, but other characters may be involved. Wilson (1910) mentions a strain of *B. typhosus*, isolated from the urine of a typhoid carrier, which had acquired the power to ferment lactose.

CHAPTER III

A CONSIDERATION OF THE EVIDENCE

BEFORE discussing in detail instances of variation and of "transmutation," it is necessary to consider the value of the evidence offered in support of them and the possible *sources of error*, in the way of both observation and deduction.

1. First and foremost must be considered the possibilities of *contamination*. A single colony, even after repeated subculture and replating, may not represent an absolutely pure culture and cannot be *proved* not to contain a single bacterium of another species, the appearance of which in greater numbers at a later stage of the experiment might suggest variation.

The importance of contamination as a source of error is so obvious that efficient precautions are taken in almost all cases to eliminate it.

Barber (1908) has described a method by which a strain can be *grown from a single organism* thus ensuring the purity of the culture. By means of a glass pipette possessing an extremely fine aperture—no larger than the diameter of a yeast cell—a single organism is removed under the microscope from a culture which has been repeatedly diluted.

2. The *original infection*, however, with which the investigator is dealing may itself be a "*mixed*" one and this fact may be overlooked in two ways :

The conditions of cultivation may favour the growth of one organism and inhibit that of another, so that the first may be present in such overwhelming preponderance that the second is for the time being completely submerged, as it were, and undetected. If the conditions change, as a result either of the activity of the organisms themselves or the intervention of the investigator, the balance may be restored and may even swing in the opposite direction, so that the

second organism now predominates in numbers to such an extent that the first one is lost sight of. Such a train of events might be misinterpreted and the assumption made that variation or even transmutation had occurred. Horrocks (1911) investigated the urine of a typhoid carrier which "in certain dilutions always gave practically pure cultures" of *B. typhosus*, although *B. coli* was present in small numbers. When the urine was diluted, however, an enormous increase in the *B. coli* occurred and the *B. typhosus* rapidly disappeared. Klein (1903–4) observed the same sequence of events in the peritoneal cavity on injecting a strain of *B. coli* which contained typhoid bacilli.

Smirnow (1908) quotes experiments in support of the opinion that streptococci inhibit the growth of the Klebs-Loeffler bacillus, but only for a time and he explains in this way the appearance in some cases of the bacillus in what, a few hours previously, had appeared to be an almost pure culture of streptococci.

Two other instances may be given. The sputum of a patient suffering from pneumonia and a swab from the throat in a case of diphtheria may contain, in addition to the virulent organisms which cause these diseases, avirulent organisms closely resembling them—the saprophytic pneumococcus and the pseudodiphtheria bacillus respectively. The saprophytic bacteria in both cases will grow at a temperature of 20°—22° C. though the virulent types are both unable to do so. It is evident that, other things being equal, the temperature of the incubator will decide which of the two types, the virulent or the avirulent, will predominate in the culture. The other one, although actually present, may then easily be overlooked unless an alteration in the temperature gives it, in turn, the ascendancy. In the latter event the change in virulence and in other characters would have the appearance of a "variation" brought about by change of temperature. It would actually be due to a "contamination" which had been previously overlooked.

A second organism may, again, escape detection because its recognition is made dependent upon some one character

alone—such as its morphology where naked eye and microscopic appearances are relied upon, or its virulence in the case of animal inoculation, or its fermenting power when the culture is tested by being "put through the sugars." The organism may be atypical in respect to the particular character the observer depends upon for its detection, and its subsequent discovery will then lead to erroneous conclusions. A knowledge of the extent to which organisms may be atypical in one or other character is the best safeguard against such an oversight.

The most thorough identification is demanded at the conclusion of an experiment no less than at its commencement, and the strictest rules must be observed before the continuity of two forms differing from each other is regarded as established. Such continuity may be impossible to prove even when we are dealing with a "pure culture." A certain number of organisms in a pure culture may undergo variation while the rest of the strain remain true to type. From time to time, as the conditions of growth change, now the variants may predominate almost to the exclusion of the original stock, and now the original stock may predominate almost to the exclusion of the variants, so that, following the variation, reversion may appear to take place, and yet there may actually be no continuity in the latter case between the variants which are dying out and the original stock which is again asserting itself.

3. In the living tissues the possibility of *secondary invasion* must be borne in mind. For example, the leptothrix forms which McDonald (1908) describes in the spinal fluid in cerebrospinal fever, as this writer himself recognises, cannot be regarded as morphological variants of the meningococcus without definite proof of identity.

Again, the pathogenic effects in a given case must not be attributed to an organism isolated from the tissues unless adequate proof is forthcoming of its being in fact the cause and not a secondary invader.

Forbes in 1903 drew attention to the frequency with which diphtheria bacilli were to be found in the ear discharges of

patients suffering from scarlet fever. The bacilli were present in 32 out of a series of 40 cases examined and sometimes greatly outnumbered the other organisms present.

Lustgarten's bacillus (1884) in syphilis and Sanarelli's *Bac. icteroides* (1897) in the case of yellow fever may be quoted as examples of secondary invaders to the presence of which diseases were wrongly attributed, and many other instances might be given.

Bacteria may be present in healthy organs. Ford (1900) examined the liver and kidneys of healthy animals after death with the most stringent precautions against contamination and found that at least 80 per cent. contained bacteria of various kinds.

Dudgeon (1908) states that staphylococcus albus can be cultivated from the great omentum in many healthy animals and quotes many examples to show that pathogenic organisms can exist in the body for long periods without giving rise to any symptoms. Savage (1907–8) has recorded the presence of *B. Gaertner* in the intestines of healthy young calves. Zwich and Weichel (1910) found that out of 177 healthy mice, 28 contained *B. Aertryck* in their faeces.

Post mortem invasion must be guarded against, for after the death of an animal secondary infection is extremely likely to occur. Dudgeon and Sargent (1907) record a case of pneumococcal peritonitis in man, in which the peritoneal exudate one hour after death gave a pure culture of pneumococci, whereas 26 hours later *B. coli* alone could be recognised in the same exudate.

4. The *repetition of an experiment* with an identical result as regards the variation produced is valuable confirmatory evidence, particularly in the hands of different investigators. Inability to repeat the phenomenon, though by no means disproving its original occurrence, does to some extent discredit it.

5. The *constancy of the new feature*, particularly on subculture, is of importance both as enabling one to exclude various errors of observation and as indicating the fundamental character of the change. On the other hand a tendency

to revert more or less quickly to the original type on the removal of the modifying influence indicates racial stability in character and minimises the significance of the modification. This aspect of the problem will be referred to later (*vide* p. 144). It is necessary, however, to emphasise at this point the danger of assuming a change in character to be permanent because reversion has not occurred within a certain period, even a lengthy one. A strain of *B. ruber*, for example, may show no trace of colour for 12 months together under certain conditions and yet retain undiminished its power to produce pigment in more favourable circumstances (Laurent, 1890). In other cases reversion occurs without any modification in the conditions of growth but apparently spontaneously and this after long periods of time have elapsed.

6. The necessity for *perseverance* in following a particular line of investigation needs no less emphasis. Twort (1907) took two years to train a particular strain of *B. typhosus* to ferment lactose—a result which Penfold (1910 A) failed to achieve in the case of over a dozen strains after a 15 months' trial. Coplans (1909) grew a strain of *B. tetani* on a gelatin medium for 90 days before liquefaction occurred. Eyre and Washbourn (1899) found that to raise a particular strain of avirulent saprophytic pneumococci to full virulence by animal "passage" no less than 53 successive inoculations were required. Goodman (1908) in his attempts to modify by artificial selection the acid production in a strain of diphtheria bacilli, made 18 transfers before any result was perceptible.

In all these cases, if the experiments had concluded earlier, negative results might have been obtained and a claim based on this evidence for stability in character which a more prolonged investigation would have shown not to be justified.

7. *Faultless technique* is essential to accuracy in results. In carrying out agglutination tests, for example, the utmost care and patience is demanded even from a practised observer if his conclusions are to be of any real value, and much of the confusion which at present exists on the subject of agglutination is no doubt to be attributed to bad workmanship.

Accurate observation is of no less importance. For example, the particular constituent of the bacterial protoplasm which retains a stain may be unevenly distributed throughout the cell. A "solid-staining" type of bacillus may thus give rise to one exhibiting "polar staining" as in the Klebs-Loeffler bacillus and also, under certain conditions, *B. coli* and *B. typhosus*. If the demarcation between the staining and the non-staining material be very definite a bacillus showing polar staining may closely resemble a diplococcus and confusion arise unless careful observation be made.

A deceptive appearance may in the same way be produced by the uneven staining of a bacterial filament. Wilson (1906) found that *B. coli* under the influence of urea developed filamentous forms. The staining material in these filaments under certain conditions became segmented, although the organism as a whole showed no sign of segmentation, with the result that the filament presented the appearance of a chain of cocci. Ainley Walker and Murray (1904) had previously observed the same phenomenon in the filamentous forms of *B. typhosus* produced under the influence of methyl violet.

Treatment with silver nitrate may render more apparent the division of a diplococcus or a filament into individual cells.

8. In other cases where the actual technique is perfect and the recognised method is carried out in every detail, *the method itself may be at fault*; conflicting results in such circumstances would not be due to any variation in the character of the organism concerned but to such factors as the composition of the medium, the age of the culture, the time-allowance made for a "positive result" to declare itself, and so on.

A few examples will suffice to show the importance of such factors.

(*a*) *The composition of the medium.* Sugar containing media may be unsuitable on account of impure commercial sugars being used in their preparation or from their being sterilised in vessels made of certain kinds of glass (W. B. M. Martin, 1911); they may undergo decomposition during the

process of sterilisation or they may deteriorate if kept for some time before being used, and failure to guard against these sources of error may lead to discordant results.

The streptococcus pyogenes normally fails to ferment both saccharose and raffinose in broth, but it produces acidity in old media containing either of these sugars (Martin, 1908–9). Fisher (1909) on the other hand found that diphtheria and diphtheroid bacilli which gave fermentation tests readily in fresh beef serum, failed to do so if the serum were old.

This observer and Theobald Smith (1899) both state that even virulent diphtheria bacilli may fail to yield toxin if the medium in which they are growing contains more than a trace of sugar; while Williams (1902) found many strains, which were non-pathogenic when inoculated from ordinary broth, were highly toxic when inoculated from serum culture or ascitic broth.

The neutral red reaction in the case of *B. coli* not infrequently fails but Moore and Revis (1905) claim that if lactose is substituted for glucose in the broth a positive result is invariably obtained with this organism.

Glenn (1911) observes that the acidity produced in a medium by the fermentation of its carbohydrate constituents inhibits the production of indol and may account for the failure of the indol test.

Wood (1889) has stated that the presence of glycerine in a medium will prevent the liquefaction of gelatin by organisms, not by interfering with their power of fermentation but by offering them a pabulum they prefer.

Again, if the medium used in the case of fermentation tests is itself markedly alkaline, the production of acid in small quantities may be completely masked, since it merely results in a diminution in alkalinity and this requires special means of detection. Miss Peckham (1897) quotes Timpe to the effect that all albuminous bodies give an alkaline reaction to litmus and it is well known that alkaline products are formed by the breaking down of peptone, so that the use of litmus as an indicator in peptone holding material makes the alkaline reaction prominent even when a considerable quantity

of free acid is really formed. Clark (1910) states that Hofmann's bacillus produces slight but definite acidity in dextrose broth if phenol-phthalein be used as an indicator, whereas if litmus is used the reaction always appears alkaline.

(*b*) *The age of the culture* is also a factor of importance. MacConkey (1909) finds that the indol reaction in the case of *B. coli* is not given by a 2 or 3 days' culture; the latter should be nearly a week old in order to give a positive result. A young culture of *B. diphtheriae* is unable to ferment glycerine and lactose though an older culture will usually do so (Muir and Ritchie). An old culture of cholera will not liquefy gelatin (Wood, 1889). Graham Smith (1906) has pointed out that many strains of diphtheria bacilli do not grow well in broth when first isolated from the throat and therefore do not produce acidity at once.

(*c*) *The time allowance.* In the case of many sugar fermenters an incubation period of 48 or even 72 hours is required before acidity becomes apparent, and in the case of other organisms a similar "latent period" may elapse before the appearance of pigment.

Still longer observation is sometimes necessary. Klotz (1906) describes a coliform organism which did not produce indol until the 20th day. Petrusky (1889–90) showed that in the case of *B. typhosus* a certain slow fermentation of lactose does take place in litmus whey although the organism is regarded as a non-fermenter of lactose. Penfold (1910 B) states that the same organism ferments dulcite—a property usually denied to it—if the experiment is prolonged for 2 or 3 weeks. Bahr (1912) describes a dysentery bacillus of the "Flexner" type which, after 4 days incubation, only fermented mannite but fermented maltose and saccharose also, after 15 days' incubation. Wilson (1910) states that he has frequently isolated bacilli from the intestine which required from 9 to 21 days to produce acidity in lactose litmus broth and several more days to produce gas.

In other cases the change in the reaction is reversed after an interval. Thus, Bahr mentions another "Flexner" bacillus which produced a feeble acid reaction in mannite at the end

of 24 hours but after ten days incubation gave a definitely alkaline reaction.

It is obvious from these facts that the question of the time allowance is of great importance. This point will be considered further in connection with variations in fermenting power (*vide* p. 66).

9. Finally, pathological research and *clinical observation* must go hand in hand. The former, if it is divorced from the latter, is beset with dangers.

A certain patient's blood, in the laboratory, may give at one time a positive Widal's test and at another—some weeks later—fail to do so. The knowledge that on the first occasion the patient was the subject of jaundice would suggest a simple explanation (Grünbaum, 1896) for a phenomenon otherwise difficult to elucidate.

Similarly the knowledge that sodium benzoate was being administered to a patient suffering from cystitis would afford an explanation of the fact that the strain of *B. coli* contained in the urine of the patient showed a greatly diminished power of gas production in dextrose (Penfold, 1911 A).

Again, altered pathogenicity may be falsely attributed to a strain of organisms if clinical observation is neglected. A certain disease may be latent in a patient—that is to say, present without giving rise to any noticeable symptoms. The constitutional disturbances arising from infection by the organism in question may "light up" this pre-existing disease and the symptoms of the latter then be incorrectly credited to the invading organism.

CHAPTER IV

VARIATIONS IN MORPHOLOGY

VARIATIONS in morphology will be considered under three heads: (A) zoogleic forms, (B) individual organisms, (C) colonies.

A. ZOOGLEIC FORMS.

One remarkable feature of the bacteria or schizomycetes is the tendency they show when multiplying to become massed together, not indiscriminately but in an orderly arrangement, to form "zoogleae." These forms display an extraordinary diversity of shape and structure. Thus, a single bacillus—as a result of alternate elongation and division in a transverse plane—may give rise to a long filament consisting of a row of cylindrical cells placed end to end. In other cases a number of organisms may be crowded together in a round gelatinous mass, their swollen cell walls fusing to form a mucilaginous matrix in which they lie embedded for an indefinite period.

The shape and structure of these zoogleae are not fortuitous but appear to be designed in many instances to attain some definite object of advantage to the organism, and may thus form a stage in its life history. This is the case for example in the *Bacterium radicicola*, the nitrogen-fixing organism found in the nodules on the roots of leguminous plants. It first enters the root hair from the soil; it then assumes a filamentous form and—in a manner comparable to the downward growth of the pollen tube from the stigma to the ovary—pushes its way along the interior of the hair as a long slimy thread until it penetrates the tissues of the root itself.

Again the *Beggiatoa versatilis*, a vegetation often seen at the mouth of drain pipes, may be observed to send out from a whitish gelatinous ground mass, long oscillating filaments

which emerge after sundown and the next day split up into innumerable little bacteria rods (Kerner and Oliver).

The zoogleae in which bacteria are massed together are to be regarded as a resting stage in their life history. The swollen envelope or matrix in which they are embedded, and which in some cases becomes hard and chitinous, being protective in character.

It is important however to recognise the fact that these zoogleae are merely conglomerations of a number of organisms and are not, strictly speaking, individuals themselves. Too great stress must not be laid, therefore, on their formation and the changes they undergo as evidence of variation on the part of the individuals composing them.

A regiment of soldiers during manœuvres is composed of a number of individuals, all of the same kind, comparable to pathogenic bacteria. It assumes various forms from time to time—that of serried ranks when marching, a filamentous form when advancing in single file, a "square" when awaiting a cavalry charge and yet another appearance during its resting stage when bivouacked for the night. Again, a mass meeting or "demonstration" of coal miners, composed of a different type of individual—all again of the same kind, comparable to a harmless pigment-producing organism—shows quite a different formation, namely that of an irregularly shaped crowd, the units of which are arranged somewhat concentrically. There is sometimes a tendency to the formation, at the periphery, of smaller collections or nodules showing a similar concentric arrangement. There is a constant tendency on the part of a regiment or a miners' "demonstration," wherever we find them, to reproduce exactly the forms described as typical of each. A regiment of soldiers or a crowd of pitmen may be regarded as a separate entity in one sense, but neither is an individual in the sense that a tree, composed of vegetable cells, is one. There is no interdependence of one part upon another in a body of troops or a crowd. They are temporary and can be dispersed, the individual units surviving though separated from each other. Moreover the forms they assume are not invariable. A regiment of soldiers, if a certain controlling

influence is removed, or in response to a particular stimulus—such as the attraction of a boxing-match—may assume the form of an irregular crowd concentrically arranged. A certain controlling influence in the case of the miners, or a common spontaneous impulse, may result in their marching in military formation. In other words, a collection either of the pathogenic organisms or of the harmless pigment producers, may assume temporarily a formation rightly regarded as characteristic of the other; but we should be mistaken in supposing on this account that the soldiers were being transformed into miners, or *vice versa*.

The development of zoogleic forms may occur *spontaneously*, or it may be brought about *artificially*.

I. Zoogleic Forms Occurring Spontaneously.

These may represent a regular phase in the life history of the organism; on the other hand, they may occur quite irregularly as an occasional variation—either in cultures on artificial media or in the living tissues—in which case one must regard the change as representing a phase in the life history of the organism at an earlier stage in its evolution.

Perhaps the earliest account of zoogleic forms occurring in the life history of a micro-organism was that given by Ray Lankester in 1873, with reference to the non-pathogenic *Bacterium rubescens*. The units of this bacterium were observed to become aggregated into a multitude of forms, protean in their variety—stellar, globose, massive, arborescent, catenular (or chain-like), reticular, tessellate and so on. (Diagrams of each of these forms are appended to the original article.)

The *tubercle bacillus* indicates its relationship to the streptothrices by forming in old cultures a branching filament, sometimes with "clubbed" ends, while in the living tissues, under certain conditions, it gives rise to a radiating structure similar to that of the actinomyces (Muir and Ritchie).

The bacillus of *glanders*, similarly, on artificial culture may exhibit short filamentous forms, and under certain conditions

in the living tissues show branching filaments and "clubbing" (*ibid.*).

The *Klebs-Loeffler bacillus* in young cultures, on serum and agar-agar, likewise shows clubbed and branched forms.

The bacillus of *anthrax*, both on artificial media and in the living tissues, forms leptothrix-like chains or filaments. These may be observed in a three hours' culture of the bacillus in a drop of aqueous humour (Marshall Ward and Blackman, 1910).

Adami (1892) describes *B. typhosus* as forming long filaments when grown on potato. Many observers have recorded the same phenomenon in cultures of *B. coli*. For example, Ohlmacher (1902), Revis (1908) and Wilson (1908) isolated leptothrix forms of *B. coli* from the heart's blood in a case of septicaemia, from milk and from urine respectively, the organism in each case forming a dense network of branching filaments.

Ritchie (1910) isolated leptothrix forms of *B. influenzae* from the cerebrospinal fluid in certain cases of meningitis, and similar forms of the "pseudo-influenza" bacillus from the lung in pneumonia. The causal factor in two of these instances—*B. coli* isolated from urine and *B. influenzae* from the spinal fluid in meningitis—was probably the presence, in both these fluids, of urea. Connal (1910) showed that in meningitis the spinal fluid may contain as much as ·5 per cent. of urea, and Wilson (1906) showed that urea provokes the development of leptothrix forms in many organisms.

II. Zoogleic Forms artificially produced.

1. The addition of various *chemical substances* to the culture medium in which an organism grows, leads to the development in many cases of leptothrix forms.

Péju and Rajat (quoted by Wilson, 1910) observed that *salts*, and Almquist (*ibid.*) noted that *sewage*, had this effect on *B. typhosus*. Walker and Murray (1904) showed that certain *dyes*, particularly methyl violet, had the same action on *B. typhosus*, *B. coli* and the cholera organism. Wilson (1906), by adding *urea* to the culture media, obtained leptothrix forms

of *B. typhosus, B. coli, B. pyocyaneus, B. enteritidis Gaertner*, and *B. pneumoniae Friedlander*; Adami, Abbott and Nicholson, by the addition of human *saliva*, or a trace of *bile*, obtained the same result with *B. coli*. Growth in an *acid* lactose containing medium also developed filamentous forms of this organism. They quote Schmidt's observation that growth in *caustic soda* broth had the same effect.

Adami (1892) observed that *B. pyocyaneus* took the form of a filament or, in some cases, developed into close spirals and S-shaped forms under the influence of *β. naphthol, alcohol, potassium bichromate, boric acid*; and Pakes (1901) noted that the *nitrates* of sodium, potassium, ammonium, and lithium developed in the same organism filamentous forms which showed spurious branching and resembled a cladothrix.

Wasserzug (1888) found that *B. prodigiosus* formed long bacilli and spirilla if grown in the presence of *antiseptics*. *Tartaric acid* had the same effect and by prolonged growth in media containing this acid and subjection to a temperature of 50° C. subsequently for a few minutes, a race of long bacilli was obtained which retained its new character "permanently" —that is to say on its return to ordinary media.

2. The formation of zoogleae may be provoked by alteration in *temperature*. Rodet (quoted and confirmed by Adami, Abbott and Nicholson, 1899) found that a culture of *B. coli* at a temperature of 44–45° C. developed within a few hours very long filaments.

3. The *absence of oxygen* may have the same effect. Wood (1889) observed "torula" forms of the cholera bacillus when grown in bouillon anaerobically. Noguchi (1910) found that *B. bifidus communis* only exhibited its bifurcating phase in anaerobic culture.

4. Exposure to the *ultra violet rays* leads to the formation of long filaments in *B. anthracis* (Henri, 1914).

5. *Growth in the animal body* develops on the part of the *actinomyces* its characteristic rays or clubs. These are not seen in artificial cultures (Bowlby and Andrewes).

B. Morphological Variations in Individual Organisms.

Morphological variations in individual bacteria may occur as normal phases in its life history, or they may develop in response to changes in their environment.

I. Pleomorphism in the Life History.

While many bacteria are only known under certain forms and are regarded as a micrococcus, a bacterium, a bacillus or a spirillum, others are known which in the course of their development pass through several such forms and are called "pleomorphic."

The non-pathogenic *Bacterium rubescens* already mentioned (Ray Lankester, 1873) affords a good example of the various phases an individual organism may pass through in its life history—forms described as spherical, biscuit-shaped, rod-like, filamentous and acicular succeeding each other in turn[1].

Amongst pathogenic organisms that of *cholera* affords a good example, the characteristic comma-shaped vibrio or spirillum giving place to a coccus or to a straight thread (Haffkine, 1895).

In old cultures of the *meningococcus* bacillary forms make their appearance (Arkwright, 1909). Young cultures of *B. coli* show not only typical bacilli but also small oval rods and tiny coccus-like forms (Gordon, 1897). In very young cultures of *B. diphtheriae* "solid" types largely predominate, but in a few hours these give place to "granular" types (Denny, 1903).

The variations in morphology displayed by *B. diphtheriae* are many of them so characteristic as to be of value in the identification of the organism, and with this object have been classified by Westbrook, Wilson and McDaniel (1900). Similarly Gordon (1900–1) observed that the streptococcus of scarlatina was characterised by a tendency to take the form

[1] Miss M. C. W. Young has recently reported some observations of great interest in this connection, revealing a similar pleomorphism in bacteria which extended, in her experiments, over a cycle of 14 days. (*Brit. Med. Journ.* 1914, II, p. 710.)

of a spindle or rod, in which case it was difficult to distinguish it from *B. diphtheriae*. At the same time its tendency to assume a bacillary form afforded a valuable means of distinguishing it from *S. pyogenes*.

II. Morphological Variations due to Environment.

1. These may be associated with differences in *geographical distribution*. For example, Schultz (1909) found that in Cleveland, U.S.A., during the twelve months covered by the investigation, "barred" forms of the diphtheria bacillus had almost disappeared; during the same period in Boston and Providence another observer noted that "barred" forms were unusually common while "granular" forms were very rarely met with.

2. *Prolonged cultivation* may influence morphology. Mohler and Washburn (1906) found that bovine tubercle bacilli after 11 years' cultivation had become changed into the human type.

3. The *crowding together of colonies* on the surface of the medium also influences morphology. In cultures of *B. diphtheriae*, under these conditions, the change from the "solid" to the "granular" type takes place much earlier than usual (Denny, 1903).

4. *Changes in the medium* employed may lead to changes in morphology. Gordon (1900–1) published photographs showing that the streptococcus associated by Klein and himself with scarlet fever may form, on serum, rods which closely resemble the diphtheria bacillus, though in a liquid medium it grows in typical streptococcal form. This fact may afford an explanation of the observations, recorded by Duncan Forbes (1903) and others, as to the prevalence of *B. diphtheriae* in the ear discharges of patients suffering from scarlet fever, without giving rise to symptoms of diphtheria and uninfluenced by antitoxin.

Ohlmacher (1902) and other observers have called attention to the fact that streptococci from the throat in cases of tonsilitis may, on Loeffler's serum, assume the form of bacilli closely simulating *B. diphtheriae*.

Rosenow (1912–13) describes a streptococcus which developed unusual morphological and cultural characters as the result of growth in unheated milk.

B. coli in ascitic fluid and in bile may assume diplococcic form (Adami, Abbott and Nicholson, 1899). Jenner (1898) found that *B. coli* isolated from water was less thick and opaque than normal *B. coli*, this distinction disappearing, however, after growth in milk.

Changes in the *reaction* of the medium may bring about changes in morphology, bacilli giving place to cocci and diplococci and *vice versa* (Adami, 1892).

5. The *addition to the medium of various chemical substances* influences morphology. The presence of *urea* converts *Micrococcus prodigiosus* into a bacillus (Wilson, 1906), and *Bacillus Pestis* into a coccus grouped singly or in pairs or in short chains (*ibid.*). *B. enteritidis Gaertner* on *urine-agar* develops into a coccus (*ibid.*), while *B. typhosus* and *B. pyocyaneus* grown in *carbolic acid* (1 in 600), and *creosote* (1 in 1000), assume the forms of non-motile cocci or diplococci (Adami, 1892).

Deceptive appearances are sometimes produced by the unequal distribution of the staining material in an organism under conditions such as those we are discussing. A bacillus may under the microscope appear to be a diplococcus and a filament resemble closely a chain of cocci.

Haslam (1898) found that the shape of *B. coli communis* depended upon the composition of the medium in which it was growing. If the composition of the medium were changed every 24 or 48 hours the shape of the organism changed with it. He found that the bacillus was longest (in proportion to its breadth) in media rich in nitrogenous substances, such as proteid and ammonium tartrate, and shortest in glucose media to which little of such nitrogenous material had been added.

6. Exposure to *ultra violet rays* in the case of *B. anthracis* has been shown to change the bacilli to cocci and diplococci (Henri, 1914).

7. *Electrolysis.* Electrolysis may produce changes in the morphology and staining properties of bacteria. Russ, for

example, has noted the production of elongated forms of *B. coli* in urine, with altered reaction to Gram's stain, as a result of the passage of a galvanic current of $\frac{1}{15}$th m.a. strength for one hour. The modification was produced in *B. coli* present in the human bladder in a case of cystitis and also in a specimen of urine outside the body, and it persisted for many months.

8. *Symbiosis* may affect morphology. The presence of streptococci in a young culture of *B. diphtheriae* hastens the appearance of "granular" types of the latter (Denny, 1903). Smirnow (1908) found that symbiosis of *B. diphtheriae* on culture media with (*a*) a streptococcus, (*b*) the meningococcus, and (*c*) an unidentified bacillus derived from a case of acute rhinitis, led in all three cases to the appearance of coccoid involution forms of the diphtheria bacillus. The experiment in the case of the unidentified bacillus was repeated in another way, the two organisms being grown in the two compartments of a double celloidin sac which was inserted into the peritoneal cavity of a rabbit. In the place of the diphtheria bacillus he found a Gram-positive coccus which, however, on Loeffler's blood serum reverted in 24 hours. A repetition of the experiment gave exactly the same result.

Lesieur (1901, quoted by Clark, 1910) claimed that the pseudo-diphtheria bacillus may assume the morphological characters of the *Klebs-Loeffler bacillus* as a result of symbiosis with *aurococcus aureus*.

9. *Growth in the living tissues* will sometimes modify the morphology of organisms. Gorham (1901) observed that, as convalescence from diphtheria advances, "granular" types of the bacillus give place to "solid" types, and he attributes the change to the action of the body fluids of the now immune patient.

Adami, Abbott and Nicholson (1899) describe forms of *B. coli*, isolated from the liver in normal and diseased animals (cow, sheep, rabbit, guineapig) and in man, which resembled diplococci in many cases, and in others short chains of three or four cocci. Similar forms were obtained from the bile and from ascitic and peritoneal fluids, and were produced on adding

guineapig bile to culture media, and they attribute the modification to the action of the body fluids. Further, they injected *B. coli* into the circulation in rabbits and found subsequently enormous numbers of this diplococcic form of the organism in the endothelial cells lining the hepatic vessels and also in the cells of the liver, in the bile and in the kidneys. These were detected within 30 to 60 minutes of the injection. In some instances the modification was so marked that it was not possible by passage or other means to obtain complete reversion to type. In other cases after passage through guineapigs reversion took place. The change was associated with irregular staining, and with loss of motility, of fermenting power and of power to produce indol. They found that *B. typhosus* underwent a similar modification under the same circumstances.

Jenner (1898) found that the difference in morphology already mentioned in the case of *B. coli* isolated from water disappeared after passage.

Arkwright (1909) and other observers have described a micrococcus closely resembling the meningococcus, found in almost pure culture in the spinal fluid in many cases of meningitis, which is characterised by a tendency to assume a bacillary form.

Ohlmacher (1902) inoculated a guineapig with a long "granular" type of *B. diphtheriae* but the organism recovered later from the site of the inoculation proved to be of the short "solid" type. The experiment was twice repeated with the same result. In two other experiments with different strains of *B. diphtheriae* the same observer found that during "passage" through a guineapig the reverse change occurred, a short "solid" type of organism being injected into the animal and a long granular type recovered from the spleen and liver after death.

Mohler and Washburn (1906) claim that, by prolonged growth in the living tissues of a suitable animal host, one type of tubercle bacillus can be so modified with respect to its morphological characters as to become indistinguishable from another type. Baldwin (1910), however, grew a strain of the

human tubercle bacillus in the tissues of a cow for 19 months without effecting any change in it.

Gordon (1900–1) found that the tendency exhibited by *S. scarlatinae* to assume bacillary form on certain media was suppressed after passage through the guineapig but was increased in some cases after passage through the mouse.

C. Variations in Colonies.

A given organism when grown on the same kind of medium and under the same conditions always tends to produce a colony of a particular size, form and appearance. Such a colony is regarded as "typical" of the organism in question and considerable importance is attached to its character as a means of isolating the particular organism and identifying it. The substitution of a macroscopic for a microscopic appearance possesses such obvious advantages that the former is frequently made use of instead of the latter and the more constant its features are found to be, the more reliance is placed upon it. The question therefore arises, how far can the appearance of its colonies be trusted as a means of identifying an organism?

MacConkey (1909) in speaking of colonies makes two statements. (1) *The colonies of an organism may vary even on the same plate.* (2) *Organisms of quite different character may produce colonies almost identical.* Both of these statements may be confirmed by reference to a common organism such as *B. coli.*

1. It is recognised that an organism will yield different types of colonies when grown upon different kinds of media. The character of the colony depends upon the composition of the medium. If, therefore, the material of a culture plate or tube should present slight differences in composition in different parts of its surface, it is reasonable to expect slight corresponding differences in the colonies of an organism growing upon it. Such an explanation is, however, inadequate to explain the wide differences frequently observed.

Savage (1904) carried out an elaborate investigation in order to ascertain to what extent colonies of *B. coli* on gelatin conformed to the character commonly accepted as

typical of this organism. He examined 72 strains, derived from half a dozen different sources. Out of this number 50 strains formed typical colonies but many of them on further cultivation gave rise to atypical colonies, which later, however, reverted to the common type. The remaining 22 strains (30·5 per cent.) all yielded atypical colonies. Many of these colonies bore no resemblance whatever to the common type and showed no tendency to revert to it; moreover, they differed as much from each other as from the typical colony. (Photographs showing the different appearances are to be seen with the original article.) One strain (No. 160) replated—after varying intervals—eight times in the course of several months, gave rise to no less than 14 distinct types of colony, all of them atypical. In other respects the organisms proved to be in every case typical *B. coli* in pure culture. In his opinion the material from which the organism was isolated considerably influenced the type of colony formed.

2. The second statement is confirmed by the same investigator who observed that colonies whose appearance was absolutely typical of *B. coli*, might be composed of different organisms altogether. Many years previously Klein (1899–00) pointed out that it was not safe, from their appearance alone, to regard particular colonies on gelatin as those of *B. coli* or its varieties. "Such colonies," he remarked "could not without animal experiment be declared not to be the bacillus of pseudo-tuberculosis. Moreover they might be neither *B. coli* nor its varieties nor the bacillus of pseudo-tuberculosis but some totally different organism."

W. B. M. Martin (1911) has published photographs showing the different appearances presented by colonies of the gonococcus grown from the same strain and on the same media.

3. In the third place it is known that *the addition of various substances to a culture medium* will modify the character of bacterial colonies growing on it. Thus, Penfold (1911 B, C) observed that the addition to an agar medium of certain carbohydrates developed papillae on the surface of the colonies of many organisms. *B. typhosus* exhibits this papillae formation on agar containing lactose, dulcite, or

isodulcite. He found that on raffinose agar *B. paratyphoid B* strains produced papillae but *B. Aertryck* strains failed to do so, and that this difference between the two organisms was sufficiently constant to be of value in distinguishing between them. The formation of papillae indicated, in certain cases, the acquirement on the part of some members of the strain of power to ferment the carbohydrate added to the medium.

4. *Heating* a strain of organisms before subculturing them has been observed to modify the characters of the colonies formed (Bainbridge, 1903).

5. Finally, as a result of *passage*, the type of colony formed by a strain of bacteria may be modified. Thus Adami, Abbott and Nicholson (1899) injected into a rabbit a strain of typical *B. coli*. The organism recovered formed on agar colonies closely resembling those of *S. pyogenes*. By intra-peritoneal passage through three guineapigs typical *B. coli* were obtained once more.

Variaton in other morphological characters.

Detailed reference has not been made to variation in other morphological characters, such as motility, pigment formation, the development of capsules and staining properties, since these are well known to vary greatly at different times and under different conditions. A few examples of such variation will be found in the earlier sections (*vide* Chap. II).

CHAPTER V

VARIATIONS IN FERMENTING POWER

The Fermentation of Carbohydrates.

The process of fermentation in the case of the mono-saccharides or glucoses—the compounds most readily fermented by the action of bacteria—consists of two stages, (i) the splitting of the "glucoses" with the formation of acids (formic acid, lactic acid) and (ii) the conversion of the acid, by a process of hydration, into simpler substances most of them gaseous (carbon dioxide, hydrogen, methane, etc.). In the case of the di-saccharides (lactose, maltose, saccharose) an earlier stage must first be completed, namely the "inversion" of these substances into glucose. This preliminary change is not easily recognised, but the two final stages of the process are sufficiently indicated by the formation, respectively, of acid and of gas.

The power possessed by certain bacteria to bring about the fermentation of carbohydrate compounds is subject to variation to a remarkable degree. *Different strains* of the same organism may differ from each other in their "sugar" reactions. *The same strain may vary* from time to time during cultivation, apparently spontaneously. In other cases the effect can be traced to the *conditions of growth* and is found to depend upon such factors as the temperature, the presence of oxygen, the atmospheric pressure, the age of the culture, the age of the medium and its composition. The power to produce fermentation may be modified by *symbiosis*. It is sometimes altered in the case of *carriers*, after animal "*passage*" and in the course of a *disease*. New fermenting properties may be developed as a result of *prolonged cultivation in a particular "sugar,"* or by a process of "*artificial selection.*"

I. *Different strains may possess different fermenting properties.*

The typical *pneumococcus* ferments saccharose (a disaccharide), mannite (a polyatomic alcohol) and inulin (a starch). Its power to ferment inulin is a feature upon which reliance is placed in differentiating the organism from other members of the streptococcus group. Eyre, Leatham and Washbourn (1906) found that out of 14 different strains examined by them 4 failed to ferment inulin, an equal number failed to ferment mannite and 3 failed to ferment saccharose.

Strains of the *meningococcus* obtained from epidemic and from sporadic cases of meningococcal meningitis exhibit differences in their fermenting properties (Batten). Arkwright (1909) describes several strains of the meningococcus which failed to ferment any sugars—in some cases "permanently," and in other cases for varying periods after their isolation.

Wilson (1908) analyses the "sugar reactions" in the case of 44 gas producing *coliform* organisms obtained from the urine of patients suffering from cystitis and pyelitis. The various strains showed an extraordinary diversity in their fermenting properties.

Many observers have recorded marked differences in fermenting properties between different strains of *dysentery* bacilli. One example will suffice. Bahr (1912) collected 28 different strains of dysentery bacilli in Fiji. He tested the power of these strains to ferment six sugars (dextrose, dulcite, maltose, saccharose, lactose and mannite) with the result that the 28 strains formed no less than 7 distinct groups. The addition of further sugars for test purposes would no doubt have revealed still more varieties.

Arkwright (1909) mentions a strain of *gonococcus*, isolated from the urethral discharge in a case of acute gonorrhoea, which fermented glucose and maltose but not saccharose, thus resembling most strains of the meningococcus and differing from the typical gonococcus which is a non-fermenter of maltose. W. B. M. Martin (1911) describes an atypical strain of the gonococcus, isolated in pure culture from the

knee joint, which did not ferment glucose, levulose, maltose or saccharose.

Gordon (quoted Martin, 1911) when testing the "sugar reactions" of 25 strains of *micrococcus catarrhalis*, discovered 3 strains which fermented glucose, maltose, galactose and saccharose—none of which sugars are normally fermented by this organism.

Strains of *B. diphtheriae* are very variable in their action on lactose and saccharose (Graham Smith, 1906).

Klotz (1906) describes a coliform organism, quickly agglutinated by high dilutions of typhoid serum, which differed from *B. typhosus* in being able to ferment glucose, lactose and saccharose.

Many other examples might be given of differences in fermentation properties displayed by different strains of the same organism.

II. *The same strain may vary spontaneously during cultivation.*

Arkwright (1909) mentions a strain of the *meningococcus* which when first tested fermented no sugars: subsequently, throughout a period of many months, it fermented maltose only; finally, after 10 months artificial culture, it fermented both maltose and glucose. Another of his strains, on the other hand, at first fermented both maltose and glucose but later fermented neither.

Rosenow (1914) obtained a strain of haemolysing *streptococci* from the throat in a case of Scarlet fever. A culture on blood agar yielded two distinct kinds of colonies, (*a*) colonies of a haemolysing organism which fermented mannite but failed to ferment maltose and saccharose, (*b*) green producing colonies of a non-haemolysing organism which would not ferment mannite but fermented maltose and saccharose. The two strains differed also in their pathogenicity.

Andrewes and Gordon (1905–6) found that "an undoubtedly pure" strain of *staphylococcus pyogenes aureus*, which yielded a brilliantly pigmented culture, produced on subculture

colonies some of which were coloured and others white. In one experiment the coloured strain formed acid in salicin and coniferin, which neither the original strain nor the white colonies was able to accomplish.

Klotz (1906) has described a *coliform* organism which did not ferment lactose or saccharose when first isolated from water but fermented both after 48 hours' growth on the media.

Horrocks (1911) describes a strain of *B. typhosus* which after 3 days' growth on bile-salt-glucose-litmus-agar gave typical fermentation tests but a week later was found to have acquired the power to ferment lactose, dulcite and salicin.

Normally *B. typhosus* ferments glycerine. Penfold (1910 A) found that an old laboratory culture of this organism on agar, when plated out, gave some colonies which fermented glycerine and others which failed to do so even after five successive subcultures had been made into peptone water.

Many observers have recorded instances of *dysentery* bacilli acquiring during cultivation the power of fermenting sugars which previously they were unable to attack—such as (in the case of the "Shiga" organism) mannite (Torrey, 1905), the di-saccharides (Kruse, quoted by Bahr). Bahr (1912) records the loss, on the part of an atypical "Shiga" organism, of power to ferment saccharose after 6 months subculture, and maltose after 7 months subculture; the loss on the part of an atypical "Flexner" organism of power to ferment lactose after 4 months, accompanied by a temporary loss of the power to ferment maltose. He quotes records of a similar loss of power to ferment lactose after 4 years (Morgan), and maltose after 9 years (Lentz). Another strain of the "Flexner" type (after a month's subculture) produced a feebly acid reaction in mannite at the end of 24 hours but after 10 days further incubation the medium became definitely alkaline. After further cultivation for some weeks it produced acidity in mannite in 24 hours. Its action on maltose varied greatly.

Sörenson (1912, quoted by Dobell) has recorded the case of a patient, suffering from glycosuria who also developed pneumaturia. The gas formation was found to be due to a peculiar bacillus, "*B. pneumaturiae*," which had gained

access to the bladder. This organism was isolated and on cultivation was found to ferment glucose, lactose and saccharose with the production of much gas. After two years the patient recovered spontaneously from the pneumaturia, though the organism was discovered still to be present in the bladder. Cultures, however, failed to yield gas on sugar media. About a year later the strain, which had been subcultured throughout this interval, suddenly re-acquired the property of producing gas, and "shortly after" the patient commenced to suffer again from pneumaturia.

The clock-like precision with which these two strains, one on artificial media and the other in the human body, are stated to have exhibited this spontaneous variation, after the same interval of time and in spite of the difference in their respective environment, may perhaps excuse some incredulity. One suspects that further investigation would have shown the modification to exist in the sugar media employed rather than in the bacteria.

III. *Fermenting properties may be modified by the conditions of growth.*

1. *The influence of temperature.* Wilson (1910) isolated *B. typhosus* from the urine of a "carrier." At a temperature of 22° C. this organism fermented lactose litmus-agar in 2 days but at a temperature of 37° C. no acidity was produced after a month's incubation. The absence of acidity at the higher temperature might be accounted for on the supposition that the products of the proteid decomposition, at this temperature, neutralised the acid formed during the process of fermentation. Wilson proved that this was not the true explanation by estimating the amount of lactose present and showing that it had not been attacked.

Coplans (1909) mentions some strains of *B. coli* which showed the reverse phenomenon, fermenting dulcite more readily at 37° than at 20° C.

Adami has described an alteration in the fermenting properties of *B. coli communis* after subjection to a high temperature in the presence of peritoneal fluid.

2. *The influence of oxygen.* Torrey (1905) found that the power of a certain dysentery bacillus to ferment maltose was augmented by alternate aerobic and anaerobic culture.

Wilson describes an atypical *B. typhosus* which slowly fermented lactose in a litmus-broth tube but produced fermentation in 3 days when the same medium was poured into a Petri dish.

Andrewes and Horder (1906) mention a streptococcal strain which failed to ferment lactose under ordinary conditions but did so readily when grown anaerobically.

3. *The influence of atmospheric pressure.* Harden (1901) has shown that the amount of formic acid produced by *B. coli* from glucose, at the ordinary pressure of the atmosphere, is very small. Under greater pressure the yield of acid is increased, while at the same time the amount of gas formed is diminished. In other words, the final stage in the process of fermentation, which consists in the conversion of the acid into various gases, is inhibited.

4. *The age of the culture.* Older cultures of *B. diphtheriae* usually ferment both glycerine and lactose ; a young culture of the same organism can attack neither of these substances (Muir and Ritchie).

5. *The age of the medium.* The streptococcus pyogenes normally does not ferment saccharose, raffinose or salicin, but if old media be used this organism will ferment both the first two substances, and even the last named in the course of a week (Martin). On the other hand *B. diphtheriae* which gives its characteristic " sugar reactions " on fresh beef serum, fails to do so if this medium is old (Fisher, 1909).

6. *The composition of the medium.* The addition of *carbolic acid* in small quantities to the media used destroys the natural fermenting properties of many bacteria (Penfold, 1911 B).

The presence of sodium benzoate inhibits the power of *B. coli* to produce gas from dextrose, one of the most stable and fundamental differences separating the coli from the typhoid-dysentery group (Herter, 1909). Penfold (1911 C) found that many intestinal organisms (*B. coli, B. enteritidis*

Gaertner, B. paratyphosus B, etc.) by growth on monochloracetic-acid-agar, were deprived of their power to form gas from glucose and other sugars; they retained, however, their power to ferment the corresponding alcohols. He found that the variation in character was maintained, even on daily subculture, for many "generations."

7. The *source* of a strain, that is to say the nature of the medium from which it is isolated, may determine certain variations in fermenting power. Thus, Revis (1908) found that strains of *B. coli* cultivated from *milk* were frequently able to ferment saccharose. Moreover a strain obtained from cow dung, which was unable to ferment saccharose, acquired the power to do so after being grown in milk. Wilson (1909) found that many coliform organisms isolated from *urine* had lost the property of forming gas from dextrose. Adami, Abbott and Nicholson (1899) describe strains of *B. coli* grown in *ascitic fluid* as being unable to ferment glucose or dextrose broth—this power being only partially regained after three passages through the guineapig.

IV. *Symbiosis may influence fermenting power.*

Major Horrocks's experiments (1911) are of interest in this connection. He found in two experiments that a typical strain of *B. typhosus* lost its power to ferment "sugars" when grown in the presence of a strain of *B. coli* derived from the urine of a "typhoid carrier." In one case reversion in character took place on further subculture. He found, in a third experiment, that a strain of *B. typhosus* derived from the urine of a "carrier," lost both its fermenting and its agglutinating properties when grown in the diluted, filtered urine of another "typhoid carrier."

V. *Variation in fermenting power is found in organisms isolated from "carriers."*

The strain of *B. typhosus* isolated by Wilson from the urine of a "typhoid carrier" was found to have acquired the power to ferment lactose and saccharose, and the power also to produce "much gas" from mannite and maltose.

VI. *Fermenting power may be altered by "animal passage."*

Klotz (1906) isolated from water an atypical organism of the *B. coli* group. This organism, after a residence of 144 days' duration in a celluloid sac within the peritoneal cavity of a rabbit, showed a temporary loss of power to ferment glucose, saccharose and lactose. The loss was most marked in the case of lactose which was, however, again fermented in the 4th subculture into lactose broth, and also by the 8th subculture on ordinary media (agar).

Peckham (1897) introduced *B. coli* into the peritoneal cavity in sufficient numbers to set up a fatal inflammatory process. The organism, recovered on the death of the animal 4 days later, showed slight changes in fermenting power.

Horrocks (1911) describes a strain of *B. typhosus* which, in the course of cultivation, acquired the power to ferment lactose, dulcite and salicin. "Passage" through 4 guineapigs destroyed the power to ferment lactose and dulcite but after 4 further passages the power was regained.

Bahr (1912) describes experiments in which flies were fed on *dysentery* bacilli (both of the "Shiga" and of the "Flexner" type) and states that the organism recovered from the intestinal tract in several cases, "undoubtedly derived from the bacillus originally fed to the flies," gave different sugar reactions. The sugar reactions had, for 9 months previously, remained constant on repeated trials. One "Shiga" organism had acquired the power to ferment maltose. In the case of a "Flexner" organism, the power of fermenting mannite (upon which the distinction between the acid and non-acid types depends) was diminished, fermentation only occurring after 4 days' incubation. Other organisms of the "Flexner" type had acquired the power to ferment maltose and saccharose. Both types of organism on subculture reverted to their original characters in the course of several months.

Adami, Abbott and Nicholson (1899) obtained from human ascitic fluid an atypical *B. coli* which had completely lost its fermenting power. This power was restored after a series

of three intra-peritoneal passages through the guineapig. Another similar strain from ascitic fluid after several passages was still unable to produce gas from glucose or dextrose.

VII. *Variation in the fermenting power of organisms may arise during the course of a disease.*

Connal (1910) has shown that in the later, chronic, stages of cerebrospinal fever the *meningococcus* isolated from the spinal fluid has lost its power to ferment dextrose.

Adami, Abbott and Nicholson (1899) in describing diplococcic forms of *B. coli*, isolated from the ascitic fluid in cases of hepatic cirrhosis, mentions that they had lost the power of fermenting glucose and lactose broths.

VIII. *The power to ferment a "sugar" may be acquired by bacteria after prolonged growth in a medium containing that sugar.*

Hiss (1904) has described a bacillus of the *dysentery* group which acquired the power to ferment maltose after being grown for some time in a maltose medium.

Twort (1907) found that dysentery bacilli (both the "Flexner" and the "Shiga" type) which did not normally ferment saccharose, did so after cultivation in a medium containing this ingredient, and by similar means the true "Shiga Kruse" organism was induced to ferment lactose.

In the same way he found that members of the *paratyphoid* group, after prolonged cultivation on a saccharose medium, all acquired the power to ferment it, while a strain of *B. typhosus* acquired the power to ferment lactose, but only after two years' "training." The same organism could be made to ferment dulcite in a very shorter period, 2 or 3 weeks being sufficient.

Penfold (1910) trained *B. typhosus* to ferment dulcite in a period of 10 days, arabinose in 2 or 3 months and isodulcite after varying intervals. He failed however to develop new fermenting powers on the part of the same organism towards lactose after a 15 months' trial, or towards saccharose and other substances after 9 months.

Burton Bradley (1910) repeated these experiments with success as regards dulcitol and arabinose.

Penfold states that in the case of *B. typhosus* the acquirement of new fermenting properties on the part of certain individuals of the strain is indicated, in some instances, by the formation of papillae on the colonies. He observed this to take place in a medium containing dulcite or sorbite. In his opinion considerable permanency in the new fermenting power was indicated if the papillae formation arose early and without subculture.

IX. *Variation in fermenting power may be brought about by a process of Artificial Selection.*

Goodman (1908) made a series of cultures of *B. diphtheriae* in dextrose broth. From this series he selected the tube giving the greatest acidity, when titrated against a standardised soda solution, and the tube giving the lowest acidity. From each of these two tubes he made a fresh series of cultures and, after 3 days' growth at 37° C., he chose out of the more acid series the tube giving the greatest acidity, and out of the less acid series the tube giving the lowest acidity. With these two tubes he made a fresh double series of cultures and repeated the process of selection. After repeating this 36 times he obtained one culture which produced intense acidity in dextrose, and a second culture which failed to produce acidity in dextrose at all and in fact made it more alkaline. These two strains were then tested with other sugars and it was found that, in both cases, the power to ferment maltose was diminished while the power to ferment saccharose was increased. Their action on dextrin was not affected.

It is to be noted that the difference in fermenting power between these two selected strains was as great as that normally existing between *B. diphtheriae* and *B. pseudo-diphtheriae*.

This process of continued selection in opposite directions does not necessarily succeed in developing strains of extreme type. For example, Buchanan and Traux (quoted by Rettger

and Sherrick, 1911) failed to develop high and low acid forming strains of *streptococcus lacticus* and Glenn (1911) records a similar failure in the case of *B. proteus.*

The significance of variations in the "Sugar Reactions."

A consideration of the action of bacteria on carbohydrates and a comparison with the similar action of other (vegetable) cells, such as yeast, justify certain conclusions.

1. The splitting up of the carbohydrate is undoubtedly effected through the agency of *enzymes or "organised ferments,"* the functions of which are inhibited or destroyed by antiseptics, such as carbolic acid, sodium benzoate, monochlor-acetic acid.

2. The fermentation of a *particular carbohydrate* is dependent on the activity of a *particular ferment,* so that the power to ferment one carbohydrate is quite independent of the power to ferment another.

This is well illustrated by Goodman's experiments. The two strains obtained by him from a culture of *B. diphtheriae,* one with greatly increased fermenting power towards dextrose and the other with almost complete absence of such power, both showed an augmentation of their power to ferment saccharose and a diminution of their power to ferment maltose. Penfold succeeded in modifying strains of *B. coli, B. enteritidis Gaertner* and *B. Grünthal* by growing them in the presence of a certain antiseptic, with the result that they lost the power to produce gas from the sugars while still retaining the power to produce gas from the corresponding alcohols.

3. *The three stages* in the process of fermentation, namely the preliminary stage of "inversion" (in the case of the di-saccharides) and the two final stages in which acid is first formed and then split up into gases, are due to the activity of three different enzymes. Failure to produce gas may be due to the absence or the inhibition of any one of these three enzymes. Failure to produce acidity may be due

to the absence or inhibition of either the "inverting" ferment or the "acid-forming" ferment.

That the several steps in the process of fermentation result from the activity of different enzymes is suggested by the following considerations. Typhoid and dysentery bacilli never, of themselves, produce gas and cannot be made to do so by training or selection; both however produce acidity in dextrose and mannite and can be "trained" to do so in lactose.

B. coli is known to produce formic acid from glucose and then to split up the formic acid into gases; Penfold has shown that growth on monochlor-acetic-acid-agar may interfere with the "formic acid-forming" property of this organism without affecting its "formic acid-splitting" power, that is to say its power to form gas from formic acid.

4. This observer has gone still further and has shown that, during the stage of *acid production*, more than one kind of acid may be formed by an organism but that the formation of each acid is the work of a special enzyme. The inhibition of the "formic acid-forming" enzyme in the case of *B. coli* did not interfere with the production of acidity in dextrose.

5. Penfold likewise showed that the splitting up of each acid, with *the formation of gases*, was the work of a special enzyme and that an enzyme which could produce gas from one acid could not do so from another. The strain of *B. coli* which was deprived of its power to form formic acid was, on this account, deprived of its power to produce gas, for, although the organism could produce other acids (as shown by the reaction of the medium) it could not split these up. If however sodium formate was added to the medium the organism at once yielded gas; or, again, if the organism were grown in dextrose with *B. typhosus* (which possesses the power of producing formic acid from dextrose but cannot split the acid up) it once more yielded gas. It is obvious, then, that in the case of *B. coli* its "acid-splitting" enzyme is only capable of splitting up formic acid and cannot form gas from other acids.

6. The development by a strain of bacteria in contact with a certain sugar, of the power to ferment that sugar is an

example of *adaptation to environment*. If a slow fermenter of dulcite is grown in a medium containing some other sugar, such as dextrose, its power to ferment dulcite is not increased.

7. The ability to split up the sugar is apparently *an advantage to the organism* concerned. That this is actually the case is confirmed by the observation of Penfold that the appearance of acidity coincides with a very rapid and a very marked increase in the number of organisms present. So constant did he find this association of events that he regards a count of the organisms as sufficient by itself to indicate the occurrence of the variation. He found, moreover, that the addition of dulcite to peptone water containing a dulcite-fermenting strain of *B. typhosus*, rendered the medium capable of supporting a population many times greater than it was able to support alone. The addition of other sugars which the organisms could not ferment did not lead to any increase in their numbers.

The ability to ferment the sugar, even if in some cases it were not of actual benefit to the fermenting organism, might still prove of advantage to it indirectly. Marked acidity of the medium is known to be unfavourable, as a rule, to bacterial growth; but it might be expected that the acid producing individuals in a strain would be unusually resistant to the products of their own activity and that their growth would, on this account, be inhibited to a less degree than that of the non-fermenters.

8. It is easy to understand how *natural selection* will cause any character to predominate which gives the possessors of it an advantage over their fellows. This is the obvious explanation of the development by a strain of bacteria, when grown on a certain sugar, of the power to ferment that sugar.

Two phases of the phenomenon, however, call for further explanation, namely the prolonged incubation period and the shortening of this period by subculture.

9. The *incubation period* may be explained in one of three ways.

In the first place it may be regarded as a "latent period" during which changes occur in the organisms as a result of

their contact with the new sugar, such changes being preparatory to the acquisition on their part of new fermenting powers. The observation already referred to, that a very rapid increase in the number of fermenting organisms occurs simultaneously with the appearance of acidity, tends to support this hypothesis. Penfold has, however, disproved it by experiment. He observed that *B. typhosus* when grown in dulcite broth gradually acquired the power to ferment the sugar. After several days had passed, plating out on dulcite agar showed that 95 per cent. of the strain were dulcite fermenters. He found that subcultures from the *non*-fermenting colonies into dulcite broth took the same length of time as the original stock to produce fermentation, thus showing that they had not undergone any preparatory change during the first incubation period.

In the second place, if the development of the new fermenting power is dependent in the first instance upon the occurrence of a spontaneous "fluctuating" variation in the required direction, and such variations are infrequent, an interval of uncertain length must necessarily intervene between the commencement of the experiment and the appearance of the variant which is to give rise to the new strain. The fact that the length of the incubation period, whenever a certain organism is "trained" to ferment a certain sugar, is fairly constant, disposes of this argument.

In the third place, the original non-fermenting strain might contain a very few fermenting individuals, in insufficient numbers, however, to give any evidence of their presence. These few "fermenters" would possess an advantage over the "non-fermenters" and multiplying more rapidly would, in time, outnumber the latter, but a certain period would necessarily elapse before they gained the ascendancy. Inasmuch, however, as the original strain can be made in the same way to ferment a number of different sugars, the strain must, on this hypothesis, contain at one and the same time fermenters of *each* of these different sugars. Even if this be granted the hypothesis offers no explanation of the fact (illustrated by the behaviour of *B. typhosus* in dulcite and in lactose respectively) that in the

presence of one sugar the fermenters of that sugar invariably gain complete ascendancy in the course of a few days, while in the presence of another sugar the fermenters of it invariably require many months to do so.

10. The *shortening of the incubation period on subculture* is more easily explained. When a few bacteria are inoculated into a tube of broth they multiply with amazing rapidity. There is a limit, however, to the number of organisms a certain volume of the medium will support—owing not only to the using up of the food but also to the accumulation of waste products—so that after a time multiplication takes place much more slowly. Subculture into fresh medium gives a new impetus to reproduction. The "fermenters" in a mixed strain gain the ascendancy by virtue of their capacity to utilise the sugar, which enables them to multiply more rapidly than the "non-fermenters." Any factor therefore which accelerates the rate of increase of *both*, hastens the ultimate mastery of the more rapidly multiplying, that is to say, the "fermenters."

11. "*Artificial selection*" appears to be an even more powerful factor in developing a particular fermenting power than "natural selection." For example, Goodman obtained by artificial selection a strain of *B. diphtheriae* which had practically lost its power to ferment dextrose, although it had been subcultured from one dextrose media to another repeatedly over a long period.

12. When an organism has been deprived by passage or by other means, of its power to ferment a particular sugar, "*reversion*" in character occurs subsequently on ordinary media, that is to say in the absence of the particular sugar in question. (The addition of the latter hastens "reversion," as Klotz has shown, but its presence plays only a subordinate part in the process). The sequence of events in such cases suggests that the enzyme is temporarily inhibited in its action and not destroyed.

13. The sudden acquisition on the part of a strain of bacteria of power *to ferment a certain sugar with which it has not been in contact*, is more difficult to explain—particularly so when such a variation occurs after long periods, even years, of

cultivation in one medium, during which repeated examinations revealed no change in fermenting properties.

The possibility must always be considered that the strain may have been subcultured into fresh media in which the new sugar was accidentally present as an unrecognised impurity, and that the bacteria "learnt" to ferment this impurity after a preliminary "training." They would be more likely to do this if the process of subculturing were only carried out at long intervals (as might easily happen in the case of a stock culture) for this would afford time for the bacteria to exhaust the normal sugar of the medium and their survival would then depend upon their power to utilise traces of any other sugar that might be present.

The possibility also suggests itself that in a medium containing a di-saccharide (lactose, maltose, saccharose) inversion might occur to a slight extent, with the formation of traces of a simpler mono-saccharide (dextrose, galactose) which the bacteria growing in the medium would then, in the same way, "learn" to ferment.

Yet a third possibility is that the particular specimen of the "sugar" used to test the fermenting properties of a strain of bacteria may not be pure. This possible source of error is, however, more easily guarded against.

Finally—even if it be admitted that a variation in fermenting power represents an adaptation to different foodstuffs—we are forced to conclude that different members of a strain differ from one another in their powers of adaptation; for, when an apparently spontaneous variation in fermenting power occurs during cultivation, in a strain derived originally from a single bacterium, fermenting and non-fermenting individuals may be found side by side on the medium. This variation between the organisms of one and the same strain we are quite unable to explain.

The Value of the Sugar Reactions.

The unsatisfactory nature of the "sugar reactions," both as a means of identification and as a basis for classification, will be apparent from the following considerations.

1. In the first place, there is the question of the time allowance to be made. When a particular organism only produces acidity in a certain sugar at the end of an incubation period lasting several days, one is in doubt whether to regard the organism in question as a slow fermenter of that sugar or as a non-fermenter of it which has acquired a new character as the result of "training."

2. Secondly, many conditions, as we have shown, modify the normal sugar reactions and may lead to erroneous conclusions. A strain of bacteria may ferment a sugar at a temperature of 22° C. and fail to do so at 37° C.; an old culture may ferment substances which a young culture is unable to do, and so on.

3. In the third place, the composition of the medium may be responsible for conflicting results. It is almost impossible to obtain many of the carbohydrates in a pure form, and yet these are used and conclusions are based on the reactions they give. Others can be obtained pure but are then too costly for general use and the commercial "sugar" is substituted. Different specimens of the same carbohydrate, even when reasonably pure, may give different results. This is the case with the starch inulin, the fermentation of which is an important distinction between the pneumococcus and other members of the streptococcus group. The process of sterilisation is a difficult one. If subjected to too high a temperature, particularly in the presence of alkaline material, the sugar may undergo a change in composition. If the temperature is not raised sufficiently sterilisation may be incomplete. If the vessels holding the medium are not made of the best Jena glass, there is a danger of the glass yielding a considerable amount of alkali to the medium during sterilisation (W. B. M. Martin, 1911). If the various media are kept for any length of time before use, the carbohydrates may deteriorate and lead to apparently abnormal sugar reactions. Examples of this have already been given. Finally, if the medium is very alkaline in the first instance, or if it is rendered so by the decomposition of the peptone present, the acid reaction may be masked.

4. Even if the composition of the medium is beyond re-

proach, the reactions are not necessarily constant. There is a great deal of evidence to show that spontaneous variations frequently occur.

5. In any case, the classification of bacteria according to their action on certain carbohydrates is a very artificial one. Twort emphasises the fact that the difference between one organism which produces such slight acidity that the alkaline reaction of the medium completely masks it, and another organism which produces a slight but definite acid reaction, is no greater than the difference between the latter organism and a third which produces marked acidity. In the same way the difference between an organism which yields acidity in 24 hours and one which requires 48 or even 72 hours to do so, cannot be regarded as a fundamental one. It is merely a matter of degree.

6. Further, the decision as to which group of carbohydrate compounds (the sugars, the glucosides, the starches, etc.) shall constitute the test substances is a purely arbitrary one, depending not infrequently upon their cheapness and the facility with which they can be obtained. If one group of carbohydrates be chosen a certain classification will follow; if another group be selected an entirely different classification may result.

The only justification for founding a classification upon one series of experiments rather than upon the other is the fact that the classification so obtained corresponds more closely to differences brought out in other ways, such as differences in agglutination or pathogenicity.

If these other differences are inconstant and distinctions based on them have been found to be unreliable, then a series of fermentation tests designed to correspond with them is at once suspect and cannot be trusted as a final appeal. If on the other hand these other differences (in agglutination, pathogenesis, etc.) are constant and have been found to justify a certain division into "species," a series of fermentation tests which correspond to them may afford a very much simpler method of deciding to which of these species a certain organism belongs, and also of separating one species from

another by "plating out" a mixed culture on the appropriate medium.

It is this that constitutes the real value of the "sugar reactions." In other words *the tests are of more use for purposes of identification than of classification.*

How far is this conclusion borne out by a study of the relation between fermentation tests and agglutination reactions?

In some cases, *alteration in fermenting powers is not accompanied by any disturbance in the agglutination properties,* which remain constant.

For example, Twort and Penfold have both shown that the altered fermenting power on the part of *B. typhosus* towards lactose is not accompanied by any alteration in agglutination properties. The organism described by Klotz as differing from *B. typhosus* in its capacity to ferment lactose and saccharose, and in other characters, nevertheless was agglutinated by typhoid serum in high dilutions.

Bahr, in describing the altered fermenting power of dysentery bacilli, following transmission through the intestine of the fly, states that the variants displayed no alteration in agglutination properties. Lentz mentions a "Flexner" strain which after seven years' laboratory cultivation lost its power to ferment maltose but still retained its agglutination properties unchanged.

In other cases, *alteration in fermenting powers is associated with loss of agglutination properties.* For example, Wilson, in describing an organism isolated from the urine of a "typhoid carrier" and considered by him to be a derivative of *B. typhosus*, states that not only were the fermenting properties altered but the agglutination tests no longer corresponded with those of *B. typhosus.* Penfold found that colonies of *B. typhosus* which had lost the property of fermenting glycerine, showed impaired agglutinability also, though typical fermenting colonies on the same plate were normal as regards agglutination.

Horrocks observed that a strain of *B. typhosus* derived from the urine of a carrier, when grown in the diluted filtered

urine of a second "typhoid carrier," was deprived not only of its power to ferment but also its property of agglutinating in the presence of typhoid serum.

Thirdly, *classifications according to fermenting powers and according to agglutination properties give altogether different results.*

Ohno (1906), in his elaborate investigations on 74 strains of dysentery derived from different sources, found that a classification based on their different powers of fermentation did not correspond with a classification according to their agglutination reactions. Torrey describes dysentery bacilli possessing different fermenting properties but giving the same agglutination reactions. The pneumococcus is distinguished from other members of the streptococcus group by its different fermenting properties, particularly with reference to inulin; nevertheless the pneumococcus and other streptococci tend to originate common group agglutinins.

It would appear therefore that the power of producing fermentation bears no relation to agglutinability, and in the final resort, if reliance is to be placed, for purposes of *classification*, on one test the other must necessarily be discredited. As regards *identification*, however, there is this to be said—organisms immediately after their isolation from the tissues or excretions are, as a rule, more typical in their fermenting properties than the same organisms after even a short period on artificial media, whereas the reverse frequently holds true as regards their agglutination reactions. In deciding, therefore, which of the two series of tests is to be relied upon in cases where they conflict, the length of the period of cultivation is of great importance; the fermentation tests will be found most reliable in the identification of an organism in those cases where agglutination tests are least so.

It is seen then that the fermentation tests, though for purposes of classification they may prove at variance with the agglutination reactions, as a means of identification may supplement the latter.

The Value of Variations in the Sugar Reactions in the Identification of Bacteria.

There are ways in which the actual variations in the sugar reactions may be of value in identifying an organism.

In the first place, the variations observed may themselves be specific in character and so far from obscuring the identity of an organism may in some cases actually *contribute to its recognition*, in the same way that the morphological variations of *B. diphtheriae* help to establish its identity.

In the second place the variations may help to *identify the source* of the organism by indicating the nature of its recent environment. Thus, if "passage" is found to modify the fermenting power of a particular species of bacteria in a particular direction, then such modification when found to exist in a member of that species may indicate a recent animal host. Again, if growth in a certain material is known to lead to certain modified "sugar reactions" on the part of a particular species, then such modification, when it is found, may furnish a clue to the source of the organism in question. For example, it has been pointed out by several observers (Revis, 1908) that the saccharose-fermenting type of the colon bacillus is more often isolated from milk than from cow-dung and MacConkey has stated (quoted, *ibid.*) that the more prolonged the sojourn in the former medium is, the more prolonged is the power to ferment saccharose on the part of the organism. Revis also describes a strain of bacteria which failed to ferment saccharose when isolated from cow-dung, but did so after being cultured in milk. Again, strains of *B. coli* exhibiting variation in gas production are more commonly of urinary than of intestinal origin.

This question will be further considered in a later chapter (*vide* p. 144).

CHAPTER VI

VARIATIONS IN VIRULENCE

THE pathologist is apt to forget that the vast majority of bacteria are non-pathogenic, that is to say, they are harmless to man. Not only so, but the activities of many of them are as beneficial to him as those of the pathogenic bacteria are the reverse. The purification of sewage and the mineralization of dead vegetable matter, to mention only two instances of bacterial action, are processes which contribute to the health and survival of the human race no less than the processes of disease conduce to its decay.

The power to cause disease depends upon two factors. In the first place, it depends upon the ability of the organisms to become parasitic, that is to say, to invade the living tissues and live and multiply there, and, in the second place, it depends upon the result of their activity, more especially as regards the formation of poisons or toxins.

The harmless nature of most bacteria is due to the fact that they have not acquired the power of becoming parasitic. In some instances, where bacteria do succeed in invading the tissues, the result of their activity within the body is apparently harmless. Ford (1900) examined the liver and kidneys of healthy animals after death, with the most stringent precautions against contamination, and found at least 80 per cent. contained bacteria of various kinds. In other cases, though organisms fail to invade the living tissues, they nevertheless produce symptoms of disease by manufacturing toxins which are absorbed, as, for example, in puerperal sapraemia.

The results produced by the presence of bacteria in the tissues are due to a variety of causes, which include (*a*) the *metabolism* of the living organisms, that is to say, the

substances assimilated and excreted by them; (*b*) the *disintegration* of the dead organisms; (*c*) the *mechanical effect* of their presence whether living or dead; (*d*) the *response made by the living tissues* to these various stimuli.

The first factor in the production of disease—the "invasiveness" of the organism—is dependent upon the degree of "*viability*" the particular organism possesses under various conditions.

The second factor—the result of their activity within the body—may be considered under two heads:—(1) the production of particular lesions in the tissues and the exhibition of a certain train of symptoms, both more or less peculiar to the particular organism giving rise to them—phenomena which are discussed under the term "*pathogenesis*," and (2) the production of a condition of "toxaemia," resulting in a general impairment of health and leading eventually to the death of the body, which we now propose to consider under the term "*virulence*."

The virulence of organisms is known to vary under different circumstances within wide limits.

1. Thus, cases of disease which occur *at the end of an epidemic* are frequently, though not invariably, less severe than those *at the beginning*, the virulence of the infecting organism having gradually diminished during the course of the epidemic. Thomson mentions an epidemic of cerebro-spinal fever comprising 30 cases, all of which were admitted to hospital and received the same treatment; of the first 16 cases admitted all died except two, of the last 14 admitted all lived except two.

This difference in virulence might of course be apparent and not real, the lessened intensity of the disease being accounted for by a difference in the resistance of those attacked by it. The weakest individuals, who are the first to succumb to the infection, also offer the poorest defence, whereas in the later stages the less fit have already been weeded out and the more robust alone remain to be attacked, and these offer a more stubborn resistance. Such an explanation might hold good in the case of a strictly localised

outbreak—for example on board a ship or in a military camp—but in a widespread epidemic—in a crowded city, for instance,—both weak and strong individuals are exposed equally to infection as the disease extends.

2. Again, the same disease may either take the form of an epidemic or *occur sporadically* in the form of isolated cases, apparently unconnected with each other. In speaking of meningococcal meningitis, Koptik attributes the diminished infectivity of the sporadic types to the senility and weakened virulence of the organism concerned.

3. A similar diminution in virulence is observed in the case of some endemic diseases *in the course of many generations.* Bahr (1912) quotes evidence to show that in Fiji, dysentery 25 years ago was a much more virulent disease than it is at the present time. The virulence of the specific virus of syphilis has been modified considerably in those parts of Europe in which it has been prevalent for centuries, such as Spain.

Here again the development, by those exposed to infection, of increased powers of resistance or "immunity," no doubt plays a part, for when the infection is introduced from places where it has long been prevalent to places where previously it has never been met with, the disease may from the first assume a virulent type.

4. *Epidemics* of a particular disease vary in virulence *at different times* and at the same time but *in different places.* Typhoid fever is, as a rule, much less severe in England than the same disease in the tropics or in the temperate regions of South America, although the organisms, apart from the question of virulence, appear to be identical.

5. Again a *particular species* of organism may produce, *at different times,* diseased conditions widely differing in their intensity. The classical example of this is the streptococcus pyogenes which may produce at one time merely a local suppuration, at another a spreading erysipelas and at another a rapidly fatal septicaemia.

6. Many pathogenic organisms when grown *outside the body, under various abnormal conditions,* lose their virulence.

(*a*) Pasteur showed 30 years ago that *B. anthracis*, if grown at a *temperature* of 43·5° C., lost its virulence in 3 or 4 weeks. Hewlett and Knight (1897) destroyed the virulence of a strain of diphtheria bacilli by subjecting it for 17 hours to a temperature of 45° C. Muir and Ritchie state that a broth culture of the diphtheria bacillus if exposed for only one hour to a temperature of 65° C. is rendered much less toxic while a culture of the tetanus bacillus under the same conditions is deprived altogether of toxicity. The bacillus of "blackleg" can likewise be rendered innocuous by exposure to a high temperature (Mohler and Washburn, 1906).

It has been thought that recovery from infectious diseases, such as the exanthemata, might be due to the effect produced in this way on the infecting organisms by the continued fever which their presence provokes. That the increase in temperature may be a protective measure on the part of the body is suggested by the experiments of Lowey and Richter (1897), in which the resistance of rabbits to infection by the pneumococcus, the diphtheria bacillus and the hog cholera bacillus was artificially increased by injury to the corpus striatum and a consequent rise in temperature, before inoculation.

These observations do not prove that the rise in temperature lessens the virulence of the organisms. Indeed this opinion has been proved to be erroneous, in some cases at least, by the work of Leutscher (1911), who tested the virulence of pneumococci isolated from the affected area of the lung at different stages of an acute lobar pneumonia. He found that the virulence of the organisms isolated at the period immediately preceding the crisis was even *greater* than that of those isolated in the early stages of the disease.

Other observers, working along different lines, have shown that a comparatively high temperature is not necessarily inimical to virulence. Eyre, Leatham and Washbourn (1906) quote the observations of Kruse and Pansini, which their own work confirms, to the effect that the virulence of the parasitic pneumococcus is often associated with inability to grow at a temperature much below that of the body—37° C. A slightly virulent strain which would grow readily at 20° C.

could, they found, be converted by "passage" into a highly virulent strain which would not grow at a temperature below 37° C. By artificial culture the reverse change could be brought about. In one experiment a single inoculation into an animal sufficed to bring about the conversion of one type into the other, the relationship between virulence and the temperature at which growth would occur being constant.

(*b*) Another abnormal condition of growth which tends to modify the virulence of organisms is the presence of *weak antiseptics*. Thus Chamberland and Roux (quoted, Muir and Ritchie) found experimentally that *B. anthracis* lost virulence if grown on a medium to which carbolic acid had been added, in the proportion of 1 to 600, or a minute quantity of Pot. bichromate. A virulent strain of *B. diphtheriae* is promptly attenuated by the addition of iodine trichloride to the medium (Mohler and Washburn, 1906).

In the living body certain *secretions* play a similar rôle. Leutscher (1911) proved that the saliva had a bactericidal effect on the pneumococcus and he attributes the diminished virulence of the pneumococci found in the mouth to this agency. Savage showed, by experiment on himself, that the streptococcus mastitidis, which causes mastitis in cows, had its virulence greatly reduced by 2 or 3 days' residence in the mucous membrane of the human pharynx.

On the other hand the addition to a medium of certain substances may cause heightened virulence. The bacillus of "blackleg," rendered avirulent by exposure to a high temperature, has its virulence completely restored if lactic acid is added to the medium in which it is growing (Mohler and Washburn, 1906). Many organisms, also, which lose their virulence rapidly on ordinary culture media, maintain it for long periods when grown in the secretions of the body—for example, in urine (*vide* p. 19).

(*c*) The presence or absence of *oxygen* is another factor of importance. For example, Haffkine (quoted Hankin, 1892) found that the cholera spirillum lost virulence considerably when grown in a current of sterile air, while Hueppe (quoted

Adami, 1892) observed that its virulence was heightened by anaerobic growth.

Pasteur, when investigating cultures of chicken cholera, found that their virulence gradually disappeared, but he discovered that it was maintained if he grew the organisms in sealed tubes so that oxygen was excluded. By this procedure loss of moisture was likewise prevented and this fact may possibly have been of no less importance than the exclusion of air.

Other organisms which, normally, do not readily lose virulence, do so rapidly if grown in an atmosphere of compressed air (Muir and Ritchie).

Harass (1906) succeeded in growing certain "anaerobic" bacteria in the presence of air and he found that under these conditions the bacillus of malignant oedema lost virulence though the bacillus botulinus retained it. It is well known that the bacillus of diphtheria produces toxins more plentifully when there is an abundant supply of air (Clark, 1910).

(*d*) In other cases the loss of virulence on artificial cultivation is to be attributed to the influence of *sunlight*, which is known to have this effect on *B. anthracis* and other pathogenic bacteria (Marshall Ward and Blackman, 1910).

(*e*) The *reaction of the medium* may also influence virulence. Miss Peckham (1897) found that the addition of an alkali to the medium increased the virulence of a strain of *B. coli*. Undue acidity of the medium may result from the action of the organisms themselves in splitting up the carbohydrate present.

7. Practically every organism becomes less virulent *when cultivated for any length of time outside the body*, that is to say, on artificial media, even under the most favourable conditions. The common pus cocci and the pneumococcus afford good illustrations of this. The loss of virulence occurs even when the growth is abundant and it persists on sub-culture.

Possibly some of the factors responsible for this change are those just mentioned, namely differences in temperature, the presence of oxygen, exposure to sunlight, the increased

acidity of the medium. Other contributing factors are found, no doubt, in the nature of the medium itself—both as regards its *chemical* composition and its *physical* properties.

(*a*) The difference in *chemical composition* between the body fluids and laboratory media must necessarily profoundly influence the metabolism of organisms transferred from one to the other. The more closely the artificial medium used resembles chemically the body fluids, the less influence will this factor exert. For example, on solidified blood serum, or media to which blood has been added, or ascitic fluid, virulence is maintained for a greater length of time than on other media. Eyre and Washbourn (1899) found that the parasitic pneumococcus kept its virulence undiminished for a couple of months on blood agar but not on ordinary media. Anne Williams (1902) found many strains of diphtheria bacillus, which were quite non-pathogenic when inoculated from broth, were highly toxic when inoculated from serum culture or ascitic broth.

In the case of *pathological exudations* the question of chemical composition is of even greater significance, for the composition of these fluids is dependent on processes of great complexity and differs widely from that of healthy excretions, and it is apparently by virtue of this very difference that pathological exudations possess the power of maintaining the virulence of organisms growing in them. It is found in practice that the best fluid in which to preserve organisms from a pathological source unchanged for subsequent examination is normal saline to which a considerable quantity of the infected material itself has been added, whether it be blood or pus or fluid from a serous cavity or some other secretion. Horrocks took the urine of a "typhoid carrier" which was loaded with virulent bacilli and kept it for 12 months in flasks exposed to the light and frequently opened to the air. At the end of that time he found that the bacilli were as virulent as when first examined, though, when subcultured on to agar or into broth, virulence was rapidly lost. Thus, two strains of virulent typhoid bacilli from the urine of "Carrier *I*" and "Carrier *S*" failed to kill a guinea-pig, when

injected into the peritoneal cavity, after being cultivated on agar for periods of two weeks and three weeks respectively.

A striking example of the influence exerted by the culture medium is afforded by the observation that vaccination with a plague strain grown on agar will protect rats against itself but not against the same strain grown on serum (quoted Penfold, 1914).

(*b*) The artificial or "unnatural" *physical conditions* under which laboratory cultures grow are no less important. The difference between a medium of solidified blood serum and the blood circulating in the living body is not only one of chemical composition. When grown on solid media the organisms are crowded together and there must necessarily be an accumulation and concentration of acids formed from the medium, and also of excreted toxins in their immediate vicinity, which may inhibit their power to produce more of the latter substances.

If in this case the metabolism of the organisms could be "damped down," the possibly inhibitory influence of such an accumulation might be prevented. Shiga (quoted by Bahr, 1912) found that the virulence of his dysentery bacillus could be maintained for as long as 12 months by keeping the cultures at freezing temperature. Whether decreased metabolism is the true explanation in this case or not, it is impossible to say without further investigation.

In the living body, on the other hand, the products of the metabolism of the organism are dissolved and carried away and absorbed and excreted.

(*c*) Moreover, in the living tissues the presence of toxins provokes the formation or liberation of "*immune bodies*" as a protective measure on the part of the body, and it has been shown by Ainley Walker (1903) that the influence of these "immune bodies"—whatever their exact nature may be—is to heighten the virulence of the organism which led to their production. This observer states, with regard to *B. typhosus*, that "the result of growing the bacillus in its immune serum was a diminution in its agglutinability, a heightening of its virulence and an increase in its resistance to serum protection."

Substances which increase the virulence of an organism may be formed in the tissues as a result of infection by an organism of a different species. For example, the staphylococcus aureus produces more extensive local lesions if, with the organism, is injected a small quantity of the serum from a case of spreading cellulitis; if the local exudate from a cellulitis is substituted for the serum no such effect is produced, showing that the phenomenon is due to bodies formed not by the bacteria which cause the cellulitis but by the tissues. Moreover the serum from a case of spreading cellulitis has the same effect in the case of other kinds of infection—due to the pneumococcus, *B. typhosus*, the tubercle bacillus and cholera (Hektoen).

(*d*) Yet a fourth contributing factor to the loss of virulence on artificial media is the fact that *other organisms are*, as far as possible, *excluded*. The object of the investigator is to obtain a pure culture, by the use of selective media or by subculturing. In pathological secretions the primary infecting organism grows in the presence of many other saprophytic or parasitic bacteria. A typhoid stool contains a multitude of organisms in addition to Eberth's bacillus and pneumonic sputum often supplies evidence of a mixed infection. The influence of symbiosis on the virulence of organisms will be referred to later, but it is noteworthy, in this connection, that many organisms which are more prone than others to lose virulence when grown on artificial media are likewise more often found in pathological conditions associated with other organisms.

Whatever the explanation may be, the fact remains that cultures on artificial media tend to lose virulence. On the other hand if bacteria grow in pathological secretions, and particularly if they successfully invade the body of an animal and multiply in that animal's tissues, their virulence often becomes greatly increased.

8. *Pathological secretions* possess the power not only of preserving virulence but of actually developing that property on the part of organisms growing in them. For example, the comparatively harmless *B. coli communis*, present in large

numbers in the healthy intestine, develops in many inflammatory conditions of the intestinal mucous membrane the property of virulence. Sanarelli (1894) caused the colon bacillus to become virulent in experimental typhoid fever by producing an inflammation with typhoid toxin. Acute peritonitis due to *B. coli*, following strangulation of the gut—a sequence frequently observed clinically—would appear to depend upon something more than a lowering of the vitality of the tissues, for De Klecki (quoted by Peckham, 1897) found, by experimenting on dogs, that the colon bacillus acquired virulence in the lumen of a strangulated coil of intestine. Dreyfuss has described the increased virulence of *B. coli* in intestinal disease, and Fermi and Salto (quoted, Peckham) a similar increased virulence in inflamed conditions of the intestines due to cold, bad food, etc.

Harris (1901) tested the toxicity of 29 strains of *B. coli communis* derived from various sources. Out of 15 strains obtained from "natural sources" (healthy faeces, sewage, water, milk, shellfish) only two were virulent (one of these very slightly so) whereas out of 11 strains derived from pathological secretions (pus and the stools in epidemic cholera, cholera nostras and summer diarrhoea) only one (from the last-named source) was non-virulent.

The acquirement of virulence by saprophytic organisms in the cavity of an inflamed uterus during the puerperium, is another case in point.

9. In many cases the development of virulence by organisms growing in pathological exudations is due not only to the presence of inflammatory products or to the absence of substances normally found in the healthy secretions but also to some extent, as we have already said, to the *presence of other bacteria* and their products.

In discussing the effect of *symbiosis* on organisms, reference was made (*vide* p. 22) to the fact that substances excreted by one species of bacteria may markedly influence the growth of another species. An example was there given of a strain of *B. influenzae* which would grow on a sterilised medium previously impregnated with the products of a staphylococcal

growth but which could not be made to grow otherwise (Allen, 1910).

That symbiosis is an important factor in determining not only the growth but also the virulence of a strain of bacteria, is abundantly proved both by experiment and observation.

It is said that a dog will not succumb to the infection of tetanus unless it is infected simultaneously with pyogenic cocci and in man it is recognised that the prognosis is more serious if the tetanus gains access to the body by means of a suppurating wound. Some authorities explain these facts by assuming that the tetanus bacillus can only multiply in the body in the presence of pus-forming cocci (Marshall Ward and Blackman, 1910), but analogy with other phenomena of the same kind certainly suggests that it is a question of altered virulence.

Sanarelli observes that *B. coli communis* in typhoid stools was highly virulent. Muir and Ritchie state "guinea-pigs may resist the subcutaneous injection of a certain dose of the typhoid bacillus, but if at the same time a sterilised culture of the bacillus coli be injected into the peritoneum they quickly die of general infection." These authors attribute the phenomenon to the diminished vitality of the animal, but here again analogy suggests that increased viability or heightened virulence on the part of the typhoid bacilli may be factors of no less importance.

Other examples of symbiosis influencing virulence will be given in discussing "passage."

10. The successful invasion of the animal body by bacteria, leading to their increased virulence, may be brought about experimentally.

Pasteur was the first to discover that virulence could be "exalted" by "*artificial passage*" through an animal or series of animals. Rabbits or guinea-pigs are those commonly employed for the purpose and inoculation may be made into the blood stream or the peritoneal cavity and a cultivation made subsequently from the heart's blood or the peritoneal fluid; or the organisms may—instead of being introduced directly into the peritoneum—be shut up in a closed sac which is then

inserted into the body cavity and allowed to remain there for a certain time.

By the last named method, for example, Martin (1898) increased the virulence of a slightly virulent strain of *B. diphtheriae*.

Eyre and Washbourn (1899) showed that the saprophytic pneumococcus, found in the mouths of healthy persons, could by "passage" be made as virulent as the parasitic type isolated from an acute lobar pneumonia. The number of inoculations required varied considerably in different cases. In most of the experiments a series of eight or ten rabbits sufficed. In one case virulence only reached its height after no less than 53 passages. In another case a single inoculation was sufficient to convert an avirulent organism into a highly virulent one. They noted that strains in which virulence was easily raised were able to maintain their exalted virulence on suitable media for a long time, while those strains which very slowly acquired virulence, quickly lost it on artificial culture.

Mohler and Washburn (1906) mention that the virulence of the virus of rabies is increased by passage through rabbits and that the cholera organism, after passage through guinea-pigs, becomes much more virulent towards pigeons.

Other animals than those named may be utilised for the purpose of "passage." For example, Salter (1899), by means of five successive passages through the goldfinch, raised the virulence of the "pseudo-diphtheria bacillus" sufficiently to render it fatal to guineapigs.

The process of "passage" may be even more effective if it be made to alternate with culture on ordinary media. Marmorek (quoted, Muir and Ritchie) showed that the virulence of the streptococcus was enormously increased by growing it alternately in a mixture of human blood serum and bouillon and in the body of a rabbit.

11. *Inoculation with a living or dead culture of some other organism* in many cases intensifies the result. Thus, Klein (1903–4) states that the virulence of the diphtheria bacillus is greatly increased by inoculation into an animal if

the streptococcus pyogenes is inoculated with it, but not to the same degree if the diphtheria bacillus is inoculated alone.

In this connection Miss Williams' experiments (1902) are of interest. She grew two strains of avirulent (but morphologically typical) diphtheria bacilli with a strain of virulent streptococci in broth, transplanting every three or four days for 90 successive generations, without producing any change in the virulence of either organism.

The virulence of the bacillus of malignant oedema is markedly increased if this organism is inoculated together with *B. prodigiosus*, and that of the streptococcus if it is inoculated with *B. coli communis* (Muir and Ritchie).

Klein (1903–4) also found that the injection of many organisms subcutaneously (*B. coli, B. Gaertner, B. enteritidis sporogenes*, and others) enhanced the virulence of organisms such as *B. typhosus* or *V. cholerae* growing simultaneously in the peritoneal cavity.

The exalted virulence thus produced applies to the particular species of animal employed for passage and may, as we have shown, apply to another species also—but this is not necessarily the case. Indeed, the virulence towards other species may be markedly diminished. For example, the virus of rabies becomes attenuated by passage through monkeys (Mohler and Washburn, 1906). The bacillus of swine erysipelas, isolated by Loeffler, after passage through the rabbit shows exalted virulence towards this animal but attenuated virulence towards pigs (Adami, 1892). Duguid and Burdon-Sanderson found that the virulence of anthrax bacilli for bovine animals was diminished after passage through a number of guineapigs. Pasteur found that, if swine plague were inoculated from rabbit to rabbit, the organism became more virulent for the rabbit but less virulent for pigs (Muir and Ritchie). Streptococci, on being inoculated through a series of mice, acquire increased virulence for these animals but become less virulent for rabbits (Knorr, *ibid.*).

Many other examples might be given. A familiar one is the preparation of the calf lymph for "vaccination" where

advantage is taken of the same phenomenon to attenuate the virus of smallpox.

The Significance of Variation in Virulence.

There is a mass of evidence, therefore, to show that the virulence of bacteria is very variable. What is the explanation and significance of these observations?

Andrewes, in the Horace Dobell Lecture already quoted (*vide* p. 3), described the evolution of bacteria from harmless mineral feeders into animal saprophytes and finally into parasitic organisms and showed that virulence was probably the latest property to be acquired in the process. It is an axiom in the study of evolution that the latest characteristic or function to be acquired is the most unstable and the first to be lost if retrogression occurs. Each new function acquired indicates a higher degree of specialisation. The more highly specialised the activity of an organism becomes the more intricate are the processes upon which it depends and the more readily is it—like a complicated mechanism—"put out of gear." It is recognised, for example, by alienists that in cases of mental degeneration the highest faculties, which, from their absence or rudimentary character in lower animals and on other grounds, are regarded as having been the last to be evolved, are the first, and often the only ones, to show signs of impairment. The loss of virulence by bacteria, often unaccompanied by any other alteration in the character of the organism is another illustration of the same phenomenon.

The process by which this property of virulence is regained by a strain of bacteria is exactly similar to the process by which it was originally acquired by the race, but accelerated, that is to say it is a case of the survival of the fittest.

The fate however of a band of soldiers raiding an enemy's country depends not only on their numbers and strength but also on their resourcefulness, so that the "fittest" in this connection must be interpreted to mean not necessarily the strongest or most robust but those best able to protect themselves; and since the most successful method of resistance

is to attack, certain of these minute organisms acquire the power of manufacturing toxins which weaken the defence and counteract the opposition of the living tissues and so enable them to gain a firmer foothold there. It is the individuals which thus accommodate themselves to the exigencies of their surroundings that are perpetuated by a process of natural selection.

That it is a case of particular adaptation to environment and not merely a question of vitality or robustness is shown by the following observations.

(*a*) "Passage" through a certain species of animal while increasing the virulence for that species may actually diminish it for another species. If the process merely selected the strongest, the strain of organisms resulting should show heightened virulence for other animals also.

(*b*) The most virulent organisms are not necessarily the most robust. Eyre and Washbourn (1899) found, as Kruse and Pansini had done previously, that in the case of the pneumococcus the exact contrary was true. "The most virulent strains were those which were most delicate and sensitive in artificial cultivations and the less virulent ones were much less delicate and could grow under conditions in which the virulent ones were unable to flourish." The parasitic type required a certain reaction and temperature and special media. It would not grow if the reaction was even faintly acid or at a temperature much below that of the body and rapidly died out on agar or in broth. The saprophytic type grew luxuriantly either at 37° C. or at 20° C., in broth, agar, potato or gelatin, whether acid or alkaline, and retained its vitality for many months.

(*c*) Analogy with other processes of adaptation lends further support to the view put forward. Thus, Rettger and Sherrick (1911) have shown that by artificial selection a strain of organisms can be made unusually resistant to the action of an antiseptic such as corrosive sublimate. Penfold (1911 c) showed that natural selection might in the same way develop a special power of resistance to an antiseptic—in this case chlor-acetic acid. The last-named observer went further and demonstrated that the particular strain of organisms which

showed increased powers of resistance to chlor-acetic acid failed to show any similar increase in their power of resisting other antiseptics such as carbolic acid or formaldehyde. In other words it was a case of adaptation and not merely increased robustness.

(*d*) The acquirement of virulence during the process of passage is sometimes accompanied by other changes in the character of the organisms which are only to be accounted for by the theory of adaptation to a particular environment. For example, the parasitic pneumococcus isolated from the human lung in an acute lobar pneumonia (Eyre and Washbourn) was characterised by inability to grow except at a temperature near that of the human body. Again the avian tubercle bacillus—the particular race of tubercle virulent to birds—grows best at their body temperature which is higher than that of man, namely 43·5° C., a temperature at which human tubercle dies.

Certain difficulties are nevertheless presented by this theory of the development of virulence by natural selection.

(*a*) The first to suggest itself is the fact that toxins are "intracellular" as well as "extracellular" and, inasmuch as the intracellular toxins are not liberated until the death of the organism and its disintegration, their nature and potency can obviously have no influence on that organism's survival or perpetuation. We are not concerned, however, with one isolated bacterium's struggle for existence so much as with the fate of a host of bacteria invading the tissues, and it is no less obvious in their case that if their intracellular toxins are destructive of the vitality of the tissues the living bacteria will receive assistance from their dead and disintegrated comrades which they would not otherwise do, and this fact may determine the success of their invasion and consequently their perpetuation.

It is possible that in the case of some bacteria the toxic action of the substances set free on their disintegration is a purely physiological one—comparable to the effects produced by the absorption of extravasated blood—and not due to a special adaptation. The protective action, however, of

tuberculin and modern vaccines against specific diseases suggests that the rôle of the intracellular toxins is of greater significance than this hypothesis would admit.

(*b*) Another question that suggests itself is this: why, if successive passages increase virulence, do not infectious diseases, which are continually undergoing this process of passage, become of deadly virulence?

One explanation is that an obligatory parasite which kills its host sinks the ship it is sailing in and thereby sacrifices its own chance of survival, so that the most virulent organisms are weeded out and destroyed. Many infectious diseases, however, have not reached such a high degree of virulence that this factor can have become operative in their case.

(*c*) Yet a third difficulty in accounting for the development of virulence by natural selection is the occurrence of phenomena such as that described by Eyre and Washbourn in which an avirulent pneumococcus acquired a high degree of virulence after a single passage through an animal. The virulence so acquired was maintained for a couple of months on artificial media, much longer, that is to say, than it was found possible to maintain the same character when developed more slowly. It is difficult to conceive how, in the course of so few generations comparatively, natural selection could cause the character of virulence to predominate to such an extent. Moreover, if it did so one would expect the character to be lost with equal readiness outside the body.

The explanation may be that when avirulent and virulent pneumococci grow side by side on artificial media there is no selective action and the avirulent, being the more robust as these writers have shown, soon greatly outnumber the virulent; the latter, after subculture has been carried out several times, may be so few in number that they give no evidence of their presence. "Passage" in this case, by eliminating the avirulent, would very soon select out a pure strain of virulent organisms.

Another explanation, however, suggests itself. The change has more the aspect of an alteration in metabolism occurring as a direct response to the change in the food material provided. It is easy to imagine that, once established, such

altered metabolism might persist outside the body for some time if the substances provided for assimilation remained the same. In the case quoted, Eyre and Washbourn found that virulence was maintained only on media containing blood (blood agar) just as Anne Williams found that the virulence of diphtheria bacilli was maintained only on serum or ascitic fluid. (The possible relationship between toxicity and altered metabolism will be discussed later.)

(*d*) Another difficulty is the development of the property of virulence by organisms outside the living tissues, as, for example, by saprophytic bacteria in the intestine or in the cavity of the uterus during the puerperium. What part can adaptation or selection play in the case of these?

It is true that a saprophyte which has acquired the power of excreting toxins has thereby acquired the power also of lowering the vitality of the living cells exposed to their action, or even of killing these in cases where the superficial tissues have been injured previously. The toxic saprophyte by such means is enabled to procure fresh food-stuffs for its own use, but since it is forced to share the spoils with its non-toxic brethren this accomplishment is less a private gain than a public advantage, and hardly conduces more to its own survival than it does to theirs. It is evident, nevertheless, that a *strain* of saprophytes which developed toxic properties might survive and multiply under conditions in which a *strain* of non-toxic saprophytes would die out, so that the *strain* of saprophytes possessing the greatest toxicity would, other things being equal, stand the best chance of being perpetuated.

A saprophyte, e.g. in the uterus during the puerperium, may not only develop extreme toxicity but may actually invade the living tissues and become parasitic. Here obviously other questions are involved. One of these concerns the part played by the food-stuffs of bacteria and the effects of changes in these.

The fortunes of an invading army depend as much upon its successful victualling as upon its armament; if the former breaks down the latter is of no avail. A non-toxic saprophyte

which develops into a virulent parasite, invading the living tissues, undergoes a twofold adaptation, for it must necessarily acquire the faculty of nourishing itself upon unaccustomed food-stuffs as well as the faculty of excreting toxins. The second would be useless without the first.

What is the relation between these two faculties? Both imply altered metabolism; one involves a change in assimilation, the other a change in excretion; one necessitates the assimilation of highly organised materials, in the shape of proteid or extractives, the other consists in the excretion of complex substances which in some cases have been proved to be proteid in nature—the toxalbumins—and in others are more akin to extractives.

The possibility naturally suggests itself that the second phenomenon may be dependent upon the first, that, in some cases at least, *the excretion of toxins is the direct result of the altered assimilation*, comparable to the increased toxicity of the urine of a man on a certain diet.

This hypothesis would go far towards explaining the development of toxicity by saprophytic organisms growing in material of a highly albuminous nature and rich in extractives, in an inflamed uterus or in pathological exudations wherever found, without any actual invasion of the living tissues by the organism taking place.

Two observations are of interest in this connection.

(*a*) Miss Peckham (1897), in speaking of coliform organisms, expresses the opinion that the carbohydrate constituents of the culture medium are always attacked by the organisms present in preference to the proteid material and it is only when the supply of carbohydrate is exhausted that the proteid is made use of. She showed that if *B. typhosus* were repeatedly subcultured in peptone solution *which contained no carbohydrate* it acquired the power to split up the proteid and produced indol. She also quotes Péré to the effect that the appearance of the indol reaction (which depends upon the breaking up of the proteid by the organisms) is proof of the absence of carbohydrate, an opinion she herself confirmed by experiment. She found that indol formation

which followed the elimination of the sugar (lactose) by the bacteria was inhibited by its further addition.

Glenn (1911) sought to ascertain by experiment whether this inhibition in indol formation was due to the acid produced by the splitting up of the sugar. He found, however, that even when the acid was neutralised indol was not formed until all the sugar had been eliminated.

It is interesting to note that, as long ago as 1889, Cartwright Wood explained the fact that indol formation did not take place in the presence of glycerine, on the ground—not that the glycerine interfered with the activity of the bacteria—but that the glycerine offered them a pabulum which they preferred.

(*b*) The second observation is that in the case of some organisms—for example *B. diphtheriae* (Theobald Smith 1899, Fisher 1909)—toxins are formed in a culture only if the amount of the sugar in the medium is very small—not more than a "trace."

In one case, therefore, we find that organisms do not split up proteid material as long as they can subsist on other food-stuffs such as carbohydrates, and in the second case we find that some organisms, at any rate, do not elaborate toxins in the presence of much carbohydrate material. Both these observations lend support to the theory that the development of toxicity may result from an alteration in metabolism brought about by a change in the kind of food-stuffs available.

If once this altered metabolism is established the step is a short one from a saprophytic existence to a parasitic one. The organism now trained to feed on "vital" material has only to cross the border line between the dead and living tissues to become a virulent parasite.

The invasion of the living tissues, however, on the part of an organism, although it may necessitate the altered assimilation to which we have been attributing toxicity, does not always confer on the organism the property of virulence. For example, in a case recorded by Pansini, staphylococci were repeatedly subcultured from the blood over a period of years without there being any evidence of toxaemia.

We are forced, therefore, to the conclusion that if in some cases the formation of substances which are toxic in their action is purely a physiological process of excretion following on altered assimilation, in other cases this result is due to a special adaptation, the toxin being a *secretion* rather than an *excretion*.

The explanation of this and other kindred phenomena is, however, unsatisfactory and the suggestion has been made in many quarters that the property of virulence may be due to the action of something more or less distinct from the organism itself but grafted on or attached temporarily to it —something in the nature of a ferment or enzyme. This theory we shall discuss later (*vide* chap. XI) but in connection with it one observation will be made at this point, namely, that if the liberation or acquisition of a ferment by the organism is of advantage to it in its life struggle, it may still be regarded as an adaptation to environment and natural selection will in time cause the characteristic to predominate.

The Value of Virulence in Classification.

In concluding this section it only remains to say one word as to the value of classification according to virulence. Differences in a character so variable as we have shown virulence to be cannot, alone, be regarded as sufficient to justify a separation of bacteria into distinct "species." The inconsistency to which such a classification gives rise can best be demonstrated by examples.

For instance, we find in the throats of some patients convalescent from *diphtheria*, the so-called "carriers," bacilli indistinguishable from the Klebs-Loeffler bacillus, but in many cases non-virulent. In such cases the organism is almost certainly the lineal descendant of the original virulent infecting organism and cannot be regarded as a distinct species.

In other cases, in which no history of diphtheria can be elicited, bacilli again are found in the throat morphologically and culturally indistinguishable from the Klebs-Loeffler

bacillus but non-pathogenic. The weight of evidence is against the possibility of rendering such strains virulent by passage. A filtered broth culture, however, of these organisms will provoke the formation of diphtheria antitoxin in the horse (Arkwright, 1909) so that they must be regarded as true diphtheria bacilli although non-pathogenic.

The *pneumococcus* is found as a virulent organism in acute lobar pneumonia. It is normally present in the mouths of healthy persons in a form which is non-virulent but which can be made virulent by passage through an animal and in this case is indistinguishable from the pathogenic variety. These two forms are regarded only as varieties of the same species.

The comparatively harmless *B. coli communis*, normally present in the human intestine in health, gives place in many unhealthy and inflamed conditions of the intestine to a virulent organism which in every other respect is identical. In this case opinion is divided, some authorities regarding the toxic or pathogenic *B. coli* as a distinct species from the non-toxic *B. coli* although they possess no other distinguishing feature apart from virulence and this in the case of the former can be diminished by artificial culture and in the case of the latter increased by "passage."

The discovery of the *amoeba coli* in the intestines of healthy individuals in the tropics, where amoebic dysentery is rife, presents a similar problem for solution. Bahr (1912) found the *amoeba coli* in 30 per cent. of Fijians examined by him and Ashburn and Craig discovered it in 72 out of 100 soldiers examined in the Philippines though none of these 72 had diarrhoea or dysentery at the time or had ever been on the sick list with either of these diseases.

The *Bac. anthracoides*, discovered by Andrewes and described by Bainbridge (1903), was distinguished from the true *B. anthracis* by slight differences in the appearance of gelatin and agar cultures, in rate of growth and in motility and by its non-virulence. The observations of Savage and MacConkey, already quoted, as to the frequency with which atypical colonies of some organisms occur on gelatin, shows how

misleading such a distinction may be, and in this case, moreover, the difference in the appearance of colonies on agar could by the adoption of certain precautions be entirely eliminated. The rate of growth of organisms is always subject to variation. Slight motility was therefore the only distinguishing feature to which any importance could be attached, apart from the question of virulence. As regards the latter, experiments indicated that this property could be increased by passage. Nevertheless these observers regarded the difference in virulence as sufficiently fundamental to justify their description of the organism as a new species quite distinct from the *B. anthracis*, although spores of the latter organism were found with it.

The *Streptococcus erysipelatis* was formerly considered, on account of its greater virulence and certain minor differences, to be a distinct species from *S. pyogenes*; further investigation has however shown this opinion to be untenable.

Many other examples might be given but these will suffice to show the difficulties that arise from regarding virulence as a "specific" character.

CHAPTER VII

VARIATIONS IN PATHOGENICITY

We have shown that morphology, fermenting properties and virulence are all variable features. There remains to be considered one other character of bacteria which is of great value in their classification, namely their "pathogenicity" or their power to cause specific disease.

Under this head are to be considered, firstly, the kind of *animal* in which a particular organism can develop disease, secondly, the kind of *symptoms* caused, and thirdly, the kind of *lesions* produced by that organism's invasion of the living body.

The pathogenicity of an organism is something quite distinct from its other characters. Two organisms may possess the same morphology, the same fermenting power and the same degree of virulence and yet show a wide divergence in their pathogenicity, giving rise in the body to a totally different train of symptoms and lesions.

This character of pathogenicity derives particular value and importance from the fact that it is generally regarded as being more "fixed" than the other characters we have been discussing. Great reliance is, for this reason, placed upon resemblances and differences in pathogenicity in determining whether two organisms do or do not belong to the same species,—in fact it is regarded as constituting a final appeal in doubtful cases.

For example, Clark (1910) maintains that Hofmann's bacillus and the Klebs-Loeffler bacillus represent different species on the ground that the former when rendered virulent gives rise to different symptoms in the body.

Again, the gonococcus and the meningococcus show close resemblances in many of their characters but are readily

distinguished by their pathogenicity. W. B. M. Martin (1911) writes in this connection : "so far, in spite of the prevalence of gonorrhoea and the periodical occurrence of great epidemics of cerebrospinal fever, there is no satisfactory evidence that the gonococcus ever causes a meningitis or the meningococcus a urethritis. This is the more remarkable in that, on the one hand, gonorrhoeal metastases are common enough elsewhere and that, on the other, meningococci can frequently be isolated from the urine of cases in which there is not the slightest evidence of genito-urinary inflammation." He maintains that the explanation must be in differences in "pathogenicity" on the part of the two organisms and that such differences justify our regarding them as distinct species.

A study, however, of the pathogenicity of bacteria reveals the fact that this, like every other character they possess, is subject to variation—with respect to (i) the kind of animal affected, (ii) the kind of symptoms caused and (iii) the kind of lesions produced.

I. As regards the first of these we have already shown when speaking of virulence that the degree to which a particular organism *can cause disease in different species of animals* is subject to variation and can be artificially modified (*vide* p. 81 et seq.).

II. With regard to the second, *variability in the symptoms caused* is displayed in several ways.

1. In the first place, *the same species of organism may give rise in different cases to a totally different train of symptoms.*

In many instances the explanation is obvious. The symptoms naturally depend, to some extent, upon the particular organ, either primarily or solely, affected in each case. The toxic action of lead furnishes an analogy. Its absorption into the body is followed by symptoms of arterio-sclerosis or of peripheral neuritis or of renal disease according to whether the blood-vessels or the nerves or the kidneys are primarily affected. The pneumococcus, for example, may attack the meninges, the lungs, the pericardium, the peritoneum or a synovial membrane, and the difference in the symptoms in

each case is attributable to anatomical differences in the parts affected.

The determining factor may be in one case the route of infection, in another the lowered vitality of the particular organ attacked. In a third case neither explanation appears adequate and we are forced to conclude that the organism itself has some influence in determining the site of the disease.

Such an hypothesis is required, for example, to explain the fact that different strains of the tubercle bacillus, morphologically and culturally indistinguishable from one another, may produce in one patient phthisis, in another a tuberculous osteitis or arthritis, and in a third lupus. Here the analogy with lead poisoning or pneumococcal infection fails, for in both the latter conditions, although one system or organ may be for a time affected almost alone, the disease shows a tendency to extend to other regions of the body and to produce characteristic symptoms as each fresh region becomes involved. In the case of tuberculous infection there appear to be certain limitations. A patient with phthisis may develop meningitis or peritonitis or general tuberculosis, but it is rare for a phthisical patient to develop lupus or a tuberculous joint, or for one suffering from a tuberculous ostitis to develop either lupus or phthisis—facts which are significant when one considers how widespread are these diseases.

Nield and Dunkley (1909) quote an instance of a phthisical patient who moistened a scratch on her arm with her own saliva and developed lupus at that spot. Examples have been given by various observers (quoted Stelwagon, 1910) of lupus occurring on the hands of women employed in washing the clothes of patients with phthisis and of the same disease following such operations as ear piercing, tatooing and circumcision when performed by operators themselves suffering from phthisis, but such instances are sufficiently uncommon to be regarded as exceptional.

The contrast already referred to between the gonococcus and the meningococcus, in respect to the organs they particularly—one might almost say exclusively—attack and the

lesions to which they give rise, is no greater than the contrast existing between different strains of Koch's bacillus in the same respect; if we ascribe the contrast in the former case to specific differences in pathogenicity one is forced to ascribe it in the latter case to the same factor and acknowledge that different strains of the tubercle bacillus exhibit marked differences in pathogenicity.

There is one not unlikely fallacy which needs to be guarded against before we can with confidence attribute to an organism any unusual symptoms which appear to follow its invasion of the body. A certain disease may be latent in the patient, that is to say present without giving rise to any noticeable symptoms. The constitutional disturbances arising from infection by the organism in question may "light up" this pre-existing disease and the symptoms of the latter may then be incorrectly credited to the invading organism.

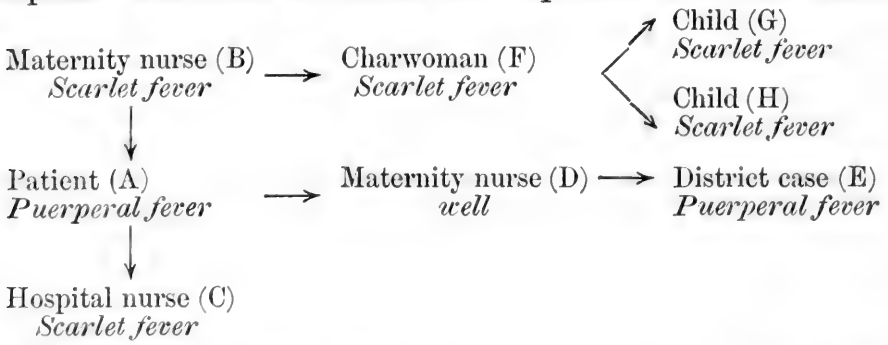
Such a sequence is well illustrated by a case recorded by Roberts and Ford under the title "A case of Cerebrospinal Fever simulating Acute Nephritis with uraemic convulsions." The patient suffered from typical symptoms of acute nephritis and uraemia—dropsy, the passage of scanty urine loaded with albumen, convulsions and coma—and showed marked improvement as a result of the treatment usually adopted for these conditions. The meningococcus was, however, isolated from the spinal fluid and the symptoms on this account attributed to that organism. In this case a pre-existing nephritis might, conceivably, have given rise at the onset of the illness to the symptoms characteristic of that disease before those characteristic of cerebrospinal fever had had time to develop; or the symptoms of the first disease might have completely masked those of the second.

2. In the second place, *one and the same strain of an organism can by artificial means be so modified as to cause an altogether different type of disease.* For example, Madame Henri (1914) found that the pathogenicity of *B. anthracis* was changed to a remarkable degree by exposure to the ultra-violet rays. Its subsequent injection into an animal produced symptoms quite unlike those caused by the normal

anthrax bacillus and it did not revert after daily subculture for two months afterwards.

3. In the third place, *a contagious disease passed from one case to another during the course of an epidemic may be characterised in different cases by widely different symptoms.*

Andrewes and Horder (1906) have recorded an example of this. A woman (A), admitted for her confinement to a maternity home, was attended by a nurse (B) who developed tonsilitis on the day the patient was delivered, and three days later was notified as a case of scarlet fever. The woman (A) developed puerperal fever and was removed to a hospital where she died. Three days after her death a nurse (C) who had attended her at the hospital developed scarlet fever. At the maternity home another nurse (D) took nurse B's place and had attended the woman (A) for a day or two before the latter's removal to hospital. Ten days later this nurse (D), who herself remained well, attended another confinement case (E) in the district. The woman (E) died of septicaemia on the fifth day after delivery. Nurse B's room, having been disinfected by the Sanitary Authority, was scrubbed by a charwoman (F). On the following day this charwoman became ill but the case was not recognised to be scarlet fever until a day or two later when her two children (G) and (H) developed typical scarlet fever and all three were removed to a fever hospital. A scheme will make the sequence of events clearer:



The original case of scarlet fever infected two other people, one with scarlet fever and the other with puerperal fever; this case of puerperal fever also infected two other people, one with puerperal fever and the other with scarlet fever.

An even more remarkable instance is recorded by Dunn

and Gordon (1905). They describe an epidemic in Hertfordshire characterised by an extraordinary diversity of symptoms in different patients. In some cases there were sneezing, coryza, and the ordinary symptoms of a common cold. In other cases patients had "aches and pains all over," stiff neck and suffered subsequently from great debility; such cases had all the appearances of influenza. In others, again, the illness closely simulated scarlet fever; it began with sore throat, rigors, vomiting, headache, fever and rapid pulse, and was accompanied by a punctate rash at the end of the first 24 hours (followed later by desquamation), the "strawberry" tongue, circumoral pallor, enlarged cervical glands which in some cases suppurated, and, in some patients, complications such as nephritis, arthritis and otorrhoea. A fourth type resembled diphtheria and exhibited a suspicious membrane on the tonsil. A fifth type was notified in some cases as typhoid fever and was characterised by epistaxis, melaena, prostration and, in some cases it is stated, a positive Widal reaction. Finally, a number of cases, particularly amongst children, resembled cerebrospinal fever and were so diagnosed; these were characterised by profuse nasal discharge, pain in the back of the neck, headache, photophobia and irritability, dilatation of one or both pupils, persistent vomiting, drowsiness, head retraction, paralysis, coma and, sometimes, convulsions and death.

Sometimes these widely divergent types were exhibited by the different members of a single family or household struck down by the disease, either simultaneously or consecutively. After a thorough investigation these observers were convinced that the outbreak of these various types of illness was due to the prevalence and spread of only *one* disease and not a number of different diseases, and a bacteriological examination of a large number of cases by Gordon showed that the disease was due to infection by an organism closely resembling, if not identical with, *M. catarrhalis*.

4. In the fourth place, *the same species of organism may give rise in different epidemics to widely different types of disease*. For example, strains of *B. influenzae* may give rise

to epidemics of "influenza" characterised by symptoms resembling in one epidemic a simple coryza, in another rheumatic fever, in a third typhoid fever, and in a fourth cerebrospinal meningitis.

5. In the fifth place, *the train of symptoms characteristic of infection by one organism may develop as a result of infection by a totally different organism.*

A striking instance of this is recorded by Head and Wilson (1899) who proved that a supposed case of rabies was actually due to infection by the diphtheria bacillus. The diagnosis of rabies was founded on the history and clinical symptoms. "The well authenticated history of a bite on the cheek by an unknown animal, the two months' incubation period, the onset with extreme pain and numbness in the region of the scar, the development of the characteristic laryngeal and respiratory spasms on attempting to take liquids, the spasm at first being slight but later more pronounced and towards the close again feeble or absent, the insomnia, the absence in the beginning of fever which later in the illness became pronounced, the rapid pulse at all stages, the attacks of violent delirium interspersed with periods of calm and complete rationality, the absence of all symptoms pointing towards any other simulating disease and the fatal termination—all serve to make an almost complete picture of rabies." The Klebs-Loeffler bacillus was isolated from the ventricular fluid and detected in the nerve cells of the medulla. The recognition of this organism was complete and beyond doubt. "Not less suggestive of rabies than the clinical history were the results of subdural inoculations of rabbits with emulsions prepared from the medulla of the patient. There occurred the long period of incubation (20 and 21 days) followed by phenomena similar to those in experimental rabies of rabbits, and other rabbits inoculated subdurally with the medulla of the first rabbits behaved in a similar manner." *B. diphtheriae* was demonstrated after death in the medulla of the rabbits. By a thorough investigation, full details of which are given, infection by the virus of rabies was definitely excluded.

Dunn and Gordon (1905, *vide supra* p. 99) have described almost typical cases of scarlet fever, of cerebrospinal fever and of influenza, which proved to be due to infection by the micrococcus catarrhalis. Gordon has described elsewhere typical cases of cerebrospinal fever due to *B. typhosus*.

Nash has recorded a remarkable case of malignant endocarditis characterised by fever, constipation, headache, drowsiness and delirium, photophobia, strabismus, head retraction and the appearance of a petechial rash. The illness, in fact, presented all the clinical features of cerebrospinal fever. A copious growth of a pure culture of the Klebs-Loeffler bacillus was obtained post mortem from the spinal fluid and a similar growth from the heart's blood. There was a history of a discharge from the ear at the beginning of the illness but no history of sore throat.

Thomson (1911) has recorded his own experience of an acute inflammation of the throat simulating diphtheria in producing, in the fourth week of the illness, temporary paralysis of the tongue, arms and legs, but proved to be due to pneumococcal infection.

Colman and Hastings (1909) state their conviction that some strains of *B. coli* are capable of causing a disease clinically identical with typhoid fever.

III. The pathogenicity of bacteria presents yet another aspect, namely the character of the *lesions* produced by them in the living tissues.

This can be studied in two ways. Firstly, by observing the lesions produced in the body at various stages in the course of an infective disease ; and secondly, by observing the lesions produced by the artificial inoculation of organisms into animals, both at the site of inoculation and elsewhere.

1. The lesions produced in the course of disease and observed post mortem not infrequently enable one to identify the infecting organism. For example, tuberculous ulceration of the intestine, tuberculous consolidation of the lungs, and tuberculous invasion of the skin, present altogether different features from typhoid ulceration of the intestine, pneumococcal

consolidation of the lung and streptococcal invasion of the skin, respectively.

It is however common experience that even in the post mortem room a certain diagnosis of the nature of the infection cannot always be made. Sydney Martin, in speaking of tuberculosis, says "There is, with the exception of the presence of the tubercle bacillus, no element in the structure of the tuberculous lesion which is diagnostic of the disease." In other words *the lesions regarded as characteristic of infection by one species of organism may be produced by infection by a totally different species.*

Such departures from what experience has taught us to regard as the normal or characteristic lesion in the case of a given organism may be accounted for by the influence of other factors beside the nature of the organism itself—such factors, for example, as the age of the patient, the route of invasion, the presence of a secondary infection, the effect of treatment, and many others. The question arises, how far, if it were possible to exclude such disturbing influences, would the lesions retain their specific character?

2. This leads us to a consideration of the second method of studying the question—by observing the lesions produced by *artificial inoculation* of animals, both at the site of inoculation and elsewhere. Such a method enables one to, so to speak, "standardise" the lesion. A healthy animal of the same species, age and weight can be utilised at each experiment, the inoculation made in the same manner, at the same site, with the same number of organisms and these of the same degree of virulence, and the animal can be killed after the same interval of time.

Many investigators maintain that under such conditions the lesions produced by a certain species of organism are constant in their appearance—that, however much the other characters of an organism may vary, this character at any rate is invariable and will establish beyond dispute to which of two species a doubtful organism actually belongs.

Thus, Klein as long ago as 1899 in describing the "bacillus of pseudo-tuberculosis" stated that in cultural and morpho-

logical characters this organism showed certain resemblances to *B. coli*. The two organisms could be distinguished from each other most certainly by animal inoculation. Subcutaneous inoculation of the first named into the guineapig gave rise to typical nodular, necrotic, purulent changes in the lymphatic glands, omentum, pancreas, liver, spleen, and lung, an effect which *B. coli* and its varieties did not produce.

Again, Shattock (and others, 1907) regards the avian tubercle bacillus and the human tubercle bacillus as two distinct species on the ground that, whereas the former when inoculated into guineapigs produces merely a local or a local and glandular disease, the latter produces visceral disease as well.

Savage (1908–9) has recorded some interesting experiments illustrating the value of animal inoculation in revealing differences in pathogenicity. He found that *streptococcus mastitidis*, which causes mastitis in the cow, was non-virulent to mice and other rodents but possessed to a marked degree the power to produce mastitis in goats when inoculated into the mammary ducts, and was thereby differentiated from *streptococcus anginosa* (isolated from human sore throat) which, though virulent to mice, did not possess the power to produce mastitis in goats. Continuing his experiments with pyogenic streptococci derived from many sources, he found that, although in their cultural properties and their virulence to mice they displayed wide differences, they all resembled each other in their inability to produce mastitis in goats. One streptococcus, for example, isolated from a fatal lymphadenitis in a boy, after it was inoculated into the teat of a goat survived for seven months as a harmless saprophyte in the milk passages.

One other example will suffice. We recognise clinically two types of pneumonia, lobar or croupous pneumonia and lobular, catarrhal or broncho-pneumonia. Both types may result from infection of the lung by the pneumococcus. The invading organism is apparently identical in the two cases, judged by the ordinary cultural and morphological tests, and the difference in the results produced are therefore attributed to differences in the age and vitality of the patient and the route of infection.

Eyre, Leatham and Washbourne (1906) endeavoured by the method of animal inoculation to ascertain whether the difference in the lesions caused depended upon specific differences in the pathogenicity of the infecting strains. They found that strains of the pneumococcus isolated from cases of lobar pneumonia when inoculated subcutaneously into the guineapig almost invariably gave rise to a local inflammatory exudation of a *fibrinous* type, whereas strains isolated from cases of broncho-pneumonia, when similarly inoculated, almost invariably gave rise to a local inflammatory exudation of a *cellular* type, easily distinguished from the other. A number of strains of pneumococci obtained from a "neutral" source, such as the mouth, likewise showed differences in the nature of the inflammatory reaction they provoked at the site of inoculation, some belonging to the "fibrinous" type and others to the "cellular" type. They further showed that this feature was not associated with any other differences between the strains as regards morphology or cultural characters or fermenting properties and was quite independent of their degree of virulence. They therefore regarded it as a specific character.

If the lesions produced in the body during the course of an infective disease are subject to variation, *are those which result from the artificial inoculation of animals any more constant?*

The materials from which to form an opinion on this point are somewhat scanty. That the feature in some cases is very constant was shown by Shattock (and others, 1907) by means of the following experiment. They grew a strain of human tubercle bacilli for eight weeks in the spleen of a pigeon. The subsequent inoculation of the organisms into a guineapig gave rise, not as might have been expected to the lesions characteristic of avian tubercle, but to those characteristic of the human type. Baldwin (1910) likewise grew the human type of tubercle bacillus for 19 months continuously in the bovine tissues without in any way affecting its pathogenic powers towards rabbits and guineapigs.

On the other hand, we have quoted in an earlier paragraph an instance of a certain strain of the diphtheria bacillus which

not only gave rise to atypical symptoms and lesions (namely those of rabies) in the human body in the course of disease but produced no less atypical lesions when inoculated into a rabbit (*vide* p. 100).

Again, Savage (1908–9) found in further experiments that a virulent strain of the streptococcus mastitidis from the udder secretion in a case of bovine mastitis, under certain conditions (namely 3 days' residence in the human pharynx), was almost deprived of its characteristic power to produce mastitis in goats.

Again, Mohler and Washburn (1906) claim that the various types of tubercle bacilli—human, bovine, avian—can be readily converted one into another, by prolonged residence in a suitable animal host, so as to be indistinguishable by the ordinary inoculation tests.

Rosenow (1914) obtained a strain of haemolysing streptococci from the throat in a case of scarlet fever. A culture on blood agar yielded two distinct kinds of colonies, (*a*) non-adherent colonies of a haemolysing organism which fermented mannite but failed to ferment maltose and saccharose, (*b*) adherent, green-producing colonies of a non-haemolysing organism which would not ferment mannite but fermented maltose and saccharose. When injected into a rabbit, the former attacked primarily the joints while the latter showed a predilection for the heart valves. In other words, the original strain on artificial cultivation gave rise to two strains which differed in their pathogenicity.

Finally, may be mentioned Foa's experiments (1890). He inoculated a rabbit with the diplococcus lanceolatus capsulatus with a fatal result. From this dead rabbit he inoculated two others, the first by injecting organisms derived from some of the fresh fibrinous pneumonic exudate in the lung, and the second by injecting organisms derived from the cerebrospinal fluid. He found that the disease set up in these two rabbits differed. The first rabbit showed, for example, an inflammatory oedema of the skin; the second did not show this. He found, however, that if the strain isolated from the lung were grown anaerobically and then injected into a rabbit the effects it

produced were indistinguishable from those produced by the strain isolated from the spinal fluid.

Whatever aspect of pathogenicity, therefore, we study, the same feature becomes apparent—namely, that this property of bacteria is, like others, subject to variation.

Variation in other Characters of Bacteria.

In the foregoing pages variations in morphology, fermenting power, virulence and pathogenesis have been discussed in detail. There remain many more characters of bacteria to be considered—such as their viability, their staining properties, their power to produce indol and to liquefy gelatin, their agglutination reactions and many others. It would be easy to illustrate the variations these characters also undergo under different conditions. Many examples will be found in Chapter II.

CHAPTER VIII

THE POSSIBLE OCCURRENCE OF TRANSMUTATION IN THE LIVING BODY

THE significance of the variations recorded in the foregoing sections, with reference to the question whether actual transmutation of bacteria can be brought about artificially or not, will be dealt with later. It is proposed, at this point, to consider another aspect of the problem, namely the possibility of transmutation occurring in the tissues of the living body.

In certain regions of the body one finds growing side by side two strains of organisms closely resembling each other in every respect save one—namely their pathogenicity. One strain is capable of causing a definite train of lesions and symptoms; the other, as a rule, does not give rise to any signs of disease. The suggestion that one strain may be in some way a derivative of the other offers a tempting hypothesis to explain both their resemblance and their proximity to each other. An illustration will, perhaps, make this clearer. In the hides of cattle may sometimes be found non-virulent bacilli closely resembling *B. anthracis*. Such an organism was discovered by Andrewes and described by Bainbridge (1903) under the name *B. anthracoides* (*vide* p. 92). The organism was stated to differ from *B. anthracis* in the appearance of its colonies, in its rate of growth, in possessing slight motility and in being non-virulent. By slightly modifying the conditions of growth, colonies on agar could be made to assume the typical appearance of anthrax colonies, while its virulence proved capable of increase by "passage." The differences in character between this organism and *B. anthracis* were deemed sufficient by these observers to justify them in classifying it as a distinct species, but it is difficult to resist the conclusion either that

the non-virulent organism was a direct derivative of the true anthrax bacillus or that it would be capable of giving rise to the latter under suitable conditions. Such a supposition is favoured, firstly, by the admission that the bundle of horse hair from which the *B. anthracoides* was isolated contained also the spores of true anthrax, and, secondly, by the discovery of Hueppe and Wood some years before (1889) of a similar non-virulent saprophytic anthrax-like organism in earth, which however on injection into a mouse rendered the animal immune to anthrax.

Similar examples of association between non-virulent and virulent organisms, otherwise closely resembling each other, may be found in the human body—in the intestine *B. coli* and *B. typhosus*, in the throat Hofmann's bacillus and the Klebs-Loeffler bacillus, in the skin the Staphylococcus epidermidis albus and the Staphylococcus pyogenes aureus, in the naso-pharynx the micrococcus catarrhalis and the meningococcus.

The exact relationship in each case has never been satisfactorily determined. Over twenty years ago Adami (quoted by Arloing, 1891) put forward the suggestion that *B. coli* might give rise in the presence of fermenting faecal matter to *B. typhosus*, a theory which has been recently revived by Tarchette (1904) and others (quoted by Hamer, 1909).

The precise relationship between the virulent *Klebs-Loeffler bacillus* and *Hofmann's bacillus* is still a matter of controversy. The latter is a harmless saprophyte not infrequently found in the pharynx of healthy persons. It is distinguished from the true diphtheria bacillus by the somewhat different appearance of its colonies on artificial media, by slight and, according to some observers, inconstant differences in its morphology and staining, by its inability to ferment glucose and other sugars, and by being non-pathogenic to man and to the guineapig. It has not been found possible to produce immunity against true diphtheria by inoculation with Hofmann's bacillus, and the injection of a filtered broth culture of the latter does not give rise to antitoxin formation in the horse (Petrie, 1905) though the filtrate in the case of even avirulent Klebs-Loeffler bacilli will do so (Arkwright, 1909). Nevertheless many in-

vestigators claim to have converted the Klebs-Loeffler type of organism into the Hofmann type—by prolonged cultivation (Lesieur, 1901), by growth at a high temperature (Hewlett and Knight, 1897), by growth in the subcutaneous tissues of an immune rat (Ohlmacher, 1902) and other methods—and maintain that the reverse change can be brought about by "passage" (Lesieur, 1901, Hewlett and Knight, 1897, Ohlmacher, 1902, etc.). Salter (1899) has stated that, by five successive passages through goldfinches, he was able to convert four strains of typical Hofmann's bacilli into no less typical Klebs-Loeffler bacilli, the transformation being complete as regards virulence, morphology and acid production, and in the power to form a toxin neutralised by diphtheria antitoxin.

Thiele and Embleton claim to have converted Hofmann's bacillus into a bacillus morphologically indistinguishable from the diphtheria bacillus and capable of secreting an exotoxin which can be neutralised by diphtheria antitoxin. This was accomplished by inoculating a succession of guineapigs with an emulsion of Hofmann's bacillus containing a certain proportion of gelatin, the organism being recovered from the peritoneal cavity after each passage.

As regards the fermenting properties of the two organisms, Clark (1910) has shown that Hofmann's bacillus does produce slight acidity in dextrose broth; while Goodman (1908), by a process of selection, obtained strains of the true diphtheria bacillus which exhibited differences in fermenting power as wide as those naturally existing between this organism and Hofmann's; and he concluded that the fermenting power was a poor guide in determining whether an organism was a pathogenic one or a harmless saprophyte.

Finally, Boycott's statistics demonstrate (Muir and Ritchie) that the period of maximal seasonal prevalence of Hofmann's bacillus immediately precedes that of true diphtheria, and Hewlett and Knight (1897) have offered evidence in support of the opinion that Hofmann's bacillus is present in increasing numbers in the throats of diphtheria patients during recovery from the disease.

Recent work by Graham Smith and others, and the inability

of these observers, on repeating the experiments of earlier investigators, to obtain the same results, somewhat invalidates the conclusions of the latter, so that the question of the possibility of a mutation between the two species remains *sub judice.*

Several species of *staphylococci* are recognised,—*S. epidermidis albus, S. pyogenes albus, S. pyogenes aureus.* The distinction between these three rests on their inequality in virulence, on their different powers of fermenting carbohydrates, and, as their names imply, on their dissimilarity in the production of pigment.

As regards virulence, the first-named organism is normally present in the skin of healthy persons and is non-pathogenic; the second possesses slight virulence, producing mild local inflammatory conditions; while the third is a virulent organism found in pathological conditions such as suppurative cutaneous and subcutaneous lesions, acute bone infection and septicaemia. *Staphylococcus epidermidis albus* may however assume a certain degree of virulence and give rise to a stitch abscess or mild inflammation (Dudgeon and Sargent, 1907) and plays an important rôle in peritonitis (*ibid.*). Andrewes and Gordon (1905–6) isolated it in pure culture in one case of otitis media and also from a boil. The *Staphylococcus pyogenes albus* can be made much more virulent by artificial passage. It has been known to become parasitic, invading the human body and circulating in the blood stream (Panichi, 1906, Southard, 1910).

In the second place, as regards their fermenting properties, Gordon (1904–5) has shown that strains of *Staphylococcus albus* isolated from the skin of healthy persons show very great diversity in their fermenting power. In an earlier paper (1903–4) he describes two strains, one a *Staph. albus* derived from the skin and the other a *Staph. pyogenes aureus* derived from pus—which, when "put through" no less than 20 carbohydrate substances, revealed different fermenting power in one only, namely mannite.

In the third place, as regards pigment formation, it has been proved by many investigators (Neumann, Dudgeon, 1908, Andrewes and Gordon, 1905–6) that non-pigmented cocci can

be obtained on culture from pigmented ones, and that cocci which fail to produce pigment under certain conditions will do so readily if the conditions are altered (*vide* p. 15). Dudgeon (1908) cites one experiment in which a *Staphylococcus aureus* was injected into an animal and a *Staphylococcus albus* was recovered from the spleen at its death. In the last case Gordon's tests were identical in both instances, showing that the character of pigment production was the only one to undergo modification, but it does not require a great stretch of imagination to suppose that just as the virulent parasitic pneumococcus and the avirulent saprophytic variety may undergo mutation (*vide* p. 82) so the highly virulent "aureus" and the less virulent "albus" might under certain circumstances be converted the one into the other.

Many more hypotheses of the same nature, and based on similar evidence, might be put forward with varying degrees of plausibility. One other example will suffice, namely the question of the relationship of the *meningococcus* to two other diplococci—*Mic. catarrhalis* on the one hand and the *pneumococcus* on the other.

The *Micrococcus catarrhalis* which is frequently present in the mouths of healthy persons, especially children, is an organism resembling in many respects the *meningococcus*, but of low virulence. The two organisms are, as a rule, easily distinguished by important differences existing between them. Thus the meningococcus is much smaller than *Mic. catarrhalis*: its colonies are also smaller and their outlines more regular; it liquefies blood serum and forms acid in dextrose, maltose and galactose which *Mic. catarrhalis* fails to do; it is more virulent also and gives rise to a different train of symptoms. The difference in size, however, is not invariable, an organism no greater than the meningococcus occasionally proving on examination to be *Mic. catarrhalis* (Hachtel and Hayward, 1911), while the appearance of a colony is a character of bacteria liable, as we have shown, to undergo great modification (*vide* p. 47).

The power of the meningococcus to produce fermentation in sugars is subject to variation. Arkwright (1909) found that

9 per cent. (out of 36 cultures) failed to produce acid in dextrose. Summers and Wilson (1909) state that out of 80 strains "nearly all" fermented the usual sugars but a few gave the fermentation reactions of *Mic. catarrhalis*. The organism isolated from sporadic cases of meningococcal meningitis shows such marked differences in its sugar reactions when compared with a typical meningococcus that some writers regard it as a distinct species (Batten). Arkwright (1909), though he refutes this, acknowledges that the sporadic type is less uniform in its fermenting powers. Some of his strains of meningococcus permanently failed to ferment any sugars; others, which failed to do so when first examined, gradually acquired the power in the course of many months; others, again, which did ferment sugars, completely lost this property after cultivation for a certain time. Another interesting fact, in this connection, is mentioned by Andrew Connal (1910), namely that in the late, chronic stages of cerebrospinal fever the meningococcus isolated from the cerebrospinal fluid is found to have lost its power to break up sugar. *Mic. catarrhalis* on the other hand may acquire power to ferment sugars. Gordon (quoted Martin, 1911) found that, out of 25 strains examined by him, three fermented dextrose, saccharose, galactose and maltose.

The meningococcus and *Mic. catarrhalis* differ in virulence but this property in the latter can be artificially raised by "passage."

As regards pathogenesis, this distinction, again, between the two organisms sometimes breaks down, symptoms typical of infection by one organism being in reality due to infection by the other. The symptoms attributable to *Mic. catarrhalis* infection differ widely. Thus it may cause an acute pharyngitis (Gordon, 1906) or a tonsilitis; it may cause a "common cold" or give rise to an infective cold and sore throat spreading from person to person (Allen, 1908); it may set up otitis media and a secondary meningitis (Barker, 1908), or, again, a primary meningitis (Arkwright and Wilson) or, finally, an epidemic so closely resembling cerebrospinal fever in its symptoms that this disease has actually been diagnosed until

a bacteriological examination demonstrated the absence of the meningococcus and the presence of *Mic. catarrhalis* (Dunn and Gordon, 1905). Conversely, the meningococcus may cause a simple coryza (*20th century Dict. of Med.*). Sometimes a "mixed infection" occurs; thus, Arkwright (1909) describes a case in which *Mic. catarrhalis* was isolated from the heart's blood after death although the typical meningococcus was proved to be present before death in the cerebrospinal fluid.

Prof. McDonald (1908) has commented upon the frequency with which, in cerebrospinal fever, leptothrix forms are found in the spinal fluid and compares this with the similar frequency of leptothrix forms in the pharynx. He considers these leptothrices to be merely secondary invaders but regards their presence as confirmatory of the opinion, now generally held, that the route of invasion of the meningococcus in cases of cerebrospinal fever is from the nasopharynx. If the appearance of these "camp followers" tends to support the opinion as to the locality from which the regiment was drawn, still further light is thrown on the question by the presence of "disbanded soldiers" in the form of non-virulent meningococci in the nasopharynx of healthy persons. Out of a total of 810 healthy persons, examined by different observers all over the world, the meningococcus was isolated from the nose in 164 cases (Hachtel and Hayward, 1911). We have already referred to the fact that organisms normally non-pathogenic may become pathogenic when growing and multiplying in inflammatory exudations (*vide* p. 79). Cerebrospinal fever is a disease more particularly of young children and it is in children that *Mic. catarrhalis* is most often discovered as an inhabitant of the pharynx in health. It has been observed that an attack of cerebrospinal fever very often commences with a purulent nasal discharge. The question arises, does this area of suppurative inflammation in the vicinity of its natural habitation afford a training ground, so to speak, for the peaceful *Micrococcus catarrhalis* preparatory to its entry upon a military career in the uniform of the meningococcus?

The oft mooted question of the relationship of the *meningococcus* to the *pneumococcus* is prompted by clinical rather than by bacteriological evidence. Pneumococcal meningitis, like all pneumococcal infections, is characterised by certain features which are also observed in meningococcal meningitis (Preble), namely an acute onset, a polymorphonuclear leucocytosis, a diminution in the chlorides in the urine, and herpes. In the second place, certain complications are common to both, namely endocarditis, pericarditis, arthritis and otitis media. In the third place, Preble observes that there is an extraordinary similarity in the seasonal distribution of the two diseases. On these grounds he suggests that the meningococcus is a variant of the pneumococcus. Certain differences between the two diseases exist. The petechial eruptions which formerly gave a name to one disease are rare in the other, but this haemorrhagic tendency is altogether absent in some epidemics of "meningococcal" meningitis. Again, "meningococcal" meningitis is a disease more especially of childhood and frequently ends in recovery; "pneumococcal" meningitis is a disease more commonly of adult life and is invariably fatal. The differences in age incidence and mortality are however compatible with the view that the causal organism is the same but of different virulence.

Finally, the sporadic nature of meningococcal meningitis, which is difficult to explain if one admits the meningococcus to be an independent organism, ceases to be so if one assumes it to be a modified form of the ubiquitous pneumococcus.

It may be argued that, although each of the several differences in character which distinguish the organisms we have been comparing, when considered by itself, may appear trivial and may prove to be variable, nevertheless all these differences, if taken together and viewed as a whole, represent a degree of divergence in type which cannot be so lightly dismissed. A series of surmises, no matter how credible these may be made to appear, does not constitute a proof. It is in our power to prove, however, that in other cases differences no less diverse in character and no less marked in degree, differences moreover which, taken together and viewed as a whole,

might be thought to represent no less wide a divergence in type, may disappear entirely under certain conditions—conditions, be it noted, precisely analogous to those which we have surmised might bring about a similar result in the cases we have been considering—namely, invasion of the living body. The experiments of Eyre, Leatham and Washbourn (1906) with strains of the pneumococcus furnish an example. These observers describe the virulent, parasitic pneumococcus as requiring for its growth a certain reaction and temperature, and particular media (blood agar); it would not grow if the reaction were even faintly acid or at a temperature much below 37° C. and rapidly died out on agar or in broth. It would not liquefy gelatin and in broth formed a dust-like deposit. The avirulent saprophytic variety, on the other hand, grew luxuriantly at temperatures ranging from 37° to 20° C.—on agar, gelatin, potato or in broth, whether acid or alkaline, slowly liquefying gelatin and producing a uniform turbidity in broth. It retained its vitality for many months. It also exhibited differences in its morphology,—"instead of isolated diplococci and streptococci large masses of cocci and diplococci were found and forms dividing into tetrads were common." Nevertheless this avirulent saprophytic pneumococcus could, by a single "passage" through a rabbit, be converted into a typical parasitic pneumococcus of high virulence.

Such a remarkable transition, if it did not actually happen, would seem to us quite as improbable as a transition from, let us say, the micrococcus catarrhalis to the meningococcus.

The purpose of this section is to suggest that a change in character, comparable to that brought about in the case of the saprophytic pneumococcus by a single animal passage, might be brought about in the case of other saprophytic organisms by an analogous process, namely by their invasion of the living body when the lowered vitality or the inflamed condition of the tissues enable them to gain a foothold therein.

CHAPTER IX

SUPPOSED INSTANCES OF TRANSMUTATION BROUGHT ABOUT EXPERIMENTALLY

I. Major Horrocks's Experiments. (*Journal of R.A.M.C.* Vol. XVI.)

In March, 1911, Major Horrocks published the records of a series of experiments of great interest. The results of these experiments may be briefly summarised as follows:

(*a*) From a strain of *B. typhosus* (derived from the urine of a carrier) he obtained, by subculture, an organism *intermediate* in its characters between *B. typhosus* and *B. coli.*

(*b*) From a second (laboratory) strain of *B. typhosus*, by symbiosis with *B. coli*, he obtained an organism which produced slight acidity in *mannite* but fermented no other sugars, and which later *reverted* to *B. typhosus.*

(*c*) From a third (laboratory) strain of *B. typhosus*, by symbiosis with the same strain of *B. coli*, he obtained an organism closely resembling *B. faecalis alcaligenes.*

(*d*) From a fourth strain of *B. typhosus* (derived from the urine of another carrier), after injection into the peritoneal cavity of a guineapig, he obtained a Gram-positive coccus resembling *streptococcus faecalis.*

(*e*) From a fifth strain of *B. typhosus* (derived from the urine of a third carrier), after injection into the peritoneal cavity of a guineapig, he obtained in three different experiments a *coliform organism* which differed widely in its fermentation and agglutination properties from *B. typhosus.*

(*f*) From a sixth strain of *B. typhosus* (derived from the stool of a fourth carrier), after growth in the diluted and filtered urine of another carrier "S," he obtained *B. faecalis*

alcaligenes. The latter organism in one experiment, after three passages through the guineapig, gave rise to *B. coli* which however reverted subsequently to *B. faecalis alcaligenes.*

(*g*) From the second (laboratory) strain of *B. typhosus* referred to above (*b*), after exposure to the same conditions, he obtained in two different experiments *B. faecalis alcaligenes.* The latter organism in two later experiments (in the first after 5 months' further growth and in the second after 8 passages through the guinea-pig) gave rise to *streptococcus faecalis*; while in a third experiment (after 18 successive passages through the guinea-pig) it gave rise to *B. coli* which, after the 19th passage, reverted to *B. faecalis alcaligenes* and this, after further "passages," in two different experiments yielded the *streptococcus faecalis.*

(*h*) From the same (laboratory) strain of *B. typhosus,* after growth in the diluted and filtered urine a different carrier "I," he obtained again *B. faecalis alcaligenes.*

To summarise these results even more concisely, it appears that Major Horrocks was forced to the conclusion that not only had an organism arisen from a strain of *B. typhosus* intermediate in character between *B. typhosus* and *B. coli,* but that other strains of *B. typhosus,* derived from three distinct sources, had in no less than five of his experiments undergone mutation into *B. faecalis alcaligenes* as a result of changes in their environment; and, further, that the *B. faecalis alcaligenes* so obtained had later, in two instances, become changed into *B. coli* (reversion taking place in both cases, however, subsequently) and, in four instances, become changed into *streptococcus faecalis,* once after prolonged cultivation and three times as the result of passage; and, finally, that one of the original strains of *B. typhosus* had undergone a similar change into *streptococcus faecalis* after passage.

Major Horrocks's statements are so startling and, if substantiated, would prove so revolutionary in character that they demand careful examination.

It may not be possible to disprove either his facts or his inferences, but it is not necessary to do so. The onus of proof

rests with the claimant. If it is possible to show that he has failed to exclude a single possible source of error, a verdict of not proven must be returned.

When considering the value of evidence adduced in support of supposed instances of variation or transmutation (*vide* Chap. III) we mentioned various sources of error. Bearing these in mind, and also the wide limits within which we have found variation may occur (*vide* Chaps. IV–VII) we will now consider in detail the processes by which Major Horrocks obtained the results he claimed and the value of the evidence he brings forward to support his contentions.

(*a*) *The alteration of* B. typhosus *to an organism intermediate between* B. typhosus *and* B. coli.

(Page 246.) A strain of *B. typhosus* was isolated from the urine of a typhoid carrier "TS"—from whose blood a pure culture of *B. typhosus* had previously been obtained. After 3 days' incubation on bile salt glucose litmus agar the strain gave the typical reactions of *B. typhosus*. At the end of a week, however, the following characters were displayed: lactose, salicin and dulcite were rendered slightly acid, broth gave a marked indol reaction, the neutral red reaction yielded a slight yellow colouration, the organism appeared only slightly motile and was *not* agglutinated by anti-typhoid serum. The organism, however, gave rise to typhoid agglutinins when injected into a rabbit, and this rabbit's serum deviated complement in the same manner as a known antityphoid serum, and the organism further had the power of absorbing the specific agglutinins from a known typhoid serum.

After 4 passages through the guineapig the organism lost its lactose fermenting property and only differed from the original *B. typhosus* by forming a trace of acid in salicin. After 4 further passages it reverted to the unusual fermenting type described.

The urine of carrier "TS" from which the strain was originally derived was again carefully tested but only typical typhoid organisms were obtained.

Criticism. We have already quoted (*vide* p. 11) instances of the occurrence of organisms, derived in some cases from

the urine of typhoid carriers, intermediate in character between *B. typhosus* and *B. coli.* Wilson (1910) described such an organism as fermenting glucose and mannite but, unlike *B. typhosus,* fermenting lactose also at 22° C. (but not at 37° C.) and failing to agglutinate with typhoid serum. The *Bacillus perturbans* of Klotz (1906), though agglutinated by high dilutions of typhoid serum, fermented lactose and saccharose, gave the neutral red reaction and produced indol.

Examples have also been given (*vide* Chapter V) to show the variability of organisms with respect to their power to ferment sugars and their ability to acquire fresh fermenting properties. *B. typhosus,* for example, may acquire the power in a few days to ferment dulcite.

The organism described here by Major Horrocks is another example of temporary variation in character with respect to the power to ferment sugars and to produce indol, associated with some modification also in agglutination properties.

(*b, c*) *The change from* B. typhosus *to* B. faecalis alcaligenes *due to symbiosis with* B. coli.

(Page 233, exp. 1.) The strain of *B. typhosus* used was a stock laboratory strain "R," from which stock vaccines were prepared—a strain, that is to say, of unimpeachable character. The strain of *B. coli* was derived from the urine of a typhoid carrier ("Bomb S"). The two organisms were added to 1 c.c. of sterilised tap water and the suspension plated. 10 days later, examination showed typical typhoid colonies and others white and opaque. The latter were planted on the usual media and in 48 hours yielded slight acidity in mannite only; no other sugars were fermented in 7 days. The original strain of *B. typhosus* used was replanted on agar and the resulting growth gave the typical reactions of this organism.

(Page 234, exp. 3.) The experiment was repeated, a different typhoid strain ("Bombay") being used. After an interval of two months, 1 c.c. of the inoculated water was added to MacConkey's bile salt broth and this plated on lactose bile salt litmus agar. A few blue colonies were seen consisting of bacilli which resembled *B. faecalis alcaligenes* in not fermenting any sugars and producing an alkaline reaction in milk.

No *B. typhosus* could be isolated and at later examinations only *B. coli* was recovered.

Criticism. Conditions of growth inimical to the life of an organism might be expected to deprive it gradually of its functions. A strain of typhoid bacilli whose vitality is at its lowest ebb would hardly be likely to ferment sugars vigorously, if at all. In both these experiments two factors were at work inimical to the life of *B. typhosus*, namely the presence of *B. coli*, and growth in water—a non-nutrient medium. After 10 days, in the first experiment, slight fermenting power persisted. After two months, in the second experiment, all fermenting power was lost. No attempt was made to resuscitate the strain of organisms on ordinary media to ascertain whether with returning vitality fermenting power would be restored.

That this explanation is the true one and that no new race of organisms was produced is suggested further by the observation that no organisms giving the ordinary reactions of *B. typhosus* survived side by side with the non-fermenters and that, at a later stage, the strain was found to have died out altogether.

(*d*) *The change from* B. typhosus *to* Streptococcus faecalis *in the peritoneal cavity of the guineapig.*

(Page 230, exp. 4.) The urine of a typhoid carrier "S" was plated and found to contain typhoid bacilli. One colony was subcultured on agar and a standard loopful of a 24 hours' growth was injected into the peritoneal cavity of a guineapig. The animal was found dead in the morning. No typhoid bacilli were found in the peritoneal fluid which contained a pure culture of a Gram-positive streptococcus giving the reactions of *S. faecalis.*

(*e*) *The change in the peritoneal cavity of a guineapig from* B. typhosus *to a coliform organism giving atypical reactions.*

The urine of a typhoid carrier "I" was plated after being kept 12 months in a flask. Two colonies of *B. typhosus* were planted on agar and labelled IB_1 and IB_2 respectively.

(*e* i) (Page 230, exp. 6.) One standard loopful of a 24 hours'

growth from the culture IB_1 was injected into the peritoneal cavity of a guineapig. The animal was found dead the next morning and a pure culture of *B. typhosus* was obtained from the heart's blood. From the peritoneal fluid and spleen was obtained, in addition to *B. typhosus*, a coliform organism possessing the following characters: a gram-negative motile bacillus, forming acid and gas in glucose, mannite, lactose and dulcite, but producing no change in salicin or cane sugar but giving rise in the neutral red medium to gas and fluorescence, not liquefying gelatin or forming indol in broth and giving an acid reaction in litmus milk without any clotting. A broth culture from the original agar slope was carefully tested but typical *B. typhosus* alone found. The broth culture was planted on agar and the experiment repeated with a loopful of this growth. The animal did not die and a pure culture of *B. typhosus* was recovered from the peritoneal cavity.

(*e* ii) (Page 230, exp. 6.) One standard loopful of a 24 hours' growth from the culture IB_2 was then injected into the peritoneal cavity of a guineapig in the same manner. The animal was found dead next morning and a pure culture of *B. typhosus* was obtained from the heart's blood and spleen. From the peritoneal fluid was obtained, in addition to *B. typhosus*, a coliform bacillus.

(*e* iii) (Page 231, exp. 7.) The urine of the typhoid carrier "I" was again plated after having been kept over 14 months in a flask. A colony was again planted on agar and a standard loopful of the growth again injected into the peritoneal fluid of a guineapig. The animal was found to be dying the next morning and was killed with chloroform and a pure culture of *B. typhosus* was obtained from the heart's blood. From the peritoneal fluid and spleen a pure culture of a coliform organism was obtained. The latter organism failed to produce any typhoid agglutinins when injected into a rabbit, or to absorb agglutinins from a known typhoid serum.

The last experiment was repeated, the same strain being used ("I") after 14 days further growth on agar. The injection did not prove fatal to the guineapig and from the peritoneal fluid a pure culture of *B. typhosus* was obtained.

Criticism. The last four experiments (*d*, *e* i, *e* ii, and *e* iii) may be discussed together.

In one experiment (*d*) *B. typhosus* derived from a carrier apparently gave rise, in the peritoneal cavity, to a Gram-positive coccus. In three experiments (*e* i, *e* ii, and *e* iii) *B. typhosus*, derived from another carrier, apparently gave rise, in the peritoneal cavity, to atypical coliform organisms.

The questions to be discussed are two—whether the strain of organisms isolated from the peritoneal cavity were derived from the original strain of *B. typhosus* injected in each case, and whether, if such continuity is established, the alteration in character is to be regarded as a temporary variation or a transmutation.

The *possibilities to be considered* are (1) whether the original strain of *B. typhosus* was pure; (2) whether the peritoneal cavity in each case was sterile before the injection was made; (3) whether it was contaminated from the skin at the time the injection was made; (4) whether it was invaded from the gut after the injection was made or after the death of the animal; (5) whether the later strain was linked up with the original one by the occurrence of reversion or the discovery of intermediate forms; (6) whether a repetition of the experiments confirmed the results; (7) whether the alteration in character falls within the recognised limits of variation discussed in the earlier part of this work.

(1) There are grounds for viewing the original cultures with suspicion. They were not made in any instance from a single organism. The urine of carrier "S" and of carrier "I," from which they were isolated, admittedly contained streptococci, *B. coli*, bacilli closely resembling *B. faecalis alcaligenes* and other coliform organisms. These other organisms were present in comparatively small numbers. In one instance it is stated that *B. coli* and *B. typhosus* were present in the proportion of 1 to 30,000. Such disparity in numbers might easily account for the less common organisms being overlooked. It is mentioned that a change in the character of the medium, brought about by simple dilution with water, enabled the associated microbes to multiply so much more rapidly than the *B. ty-*

phosus that the latter organism was soon "swamped," as it were, and disappeared altogether. If the strain of *B. typhosus* injected contained one or two specimens of a streptococcus or coliform organism, might not growth in the peritoneal cavity yield a similar result?—not before, however, some of the typhoid bacilli had succeeded in escaping from the peritoneum into the blood vessels and setting up a systemic infection. In two instances, in which the experiments were repeated, the injection of the original culture of *B. typhosus* into the peritoneal cavity did not kill the animal although a pure culture of *B. typhosus* was recovered from it. The original culture, therefore, apparently contained strains differing from each other in virulence. They may conceivably have possessed other differences.

(2) No control experiments were carried out to prove that the peritoneal cavity before the experiment was sterile. Dudgeon (1908) states that in healthy animals the omentum may normally contain the staphylococcus albus. There is evidence to show that even in healthy animals the internal organs may contain both pathogenic and non-pathogenic bacteria. Ford (1900) showed by experiments, in which rigid precautions against contamination were adopted, that the kidneys, liver and spleen of healthy animals, in a large majority of cases, contained organisms such as the staphylococcus, mesentericus, colon and paracolon bacilli, *B. subtilis* and *proteus*. In rabbits 66 per cent. of the organs examined contained bacteria, in cats over 77 per cent., in dogs over 88 per cent. In guineapigs the percentage was 77 per cent. of the organs examined and the organisms that predominated were *B. subtilis*, staphylococci and the colon bacillus. Adami, Abbott and Nicholson (1899) found in the livers of healthy animals (cows, sheep, rabbits, guineapigs) diplococci and chains of 3 or 4 cocci, which on culture yielded *B. coli* in many cases.

(3) No control experiments were conducted to exclude the possibility of skin contamination at the site of the inoculation, but such a supposition is inadequate to explain all the results obtained.

(4) The injection of organisms into the peritoneal cavity would have a threefold effect, it would make the animal ill, produce a more or less marked peritonitis, and finally kill the animal. All three events would favour the invasion of the peritoneal cavity by organisms. If the vitality of the body and consequently of the peritoneum is lowered, organisms can penetrate it from the gut even in the absence of any definite lesion or inflammation. Ford (1900) noted that in animals whose vitality was lowered, by fasting or unhealthy conditions, bacteria were more abundant in the internal organs, and that this applied particularly to bacteria of the colon type. Dudgeon and Sargent (1907) have shown that at the earliest stage of peritonitis the staphylococcus albus (either normally present on the surface of the gut or penetrating from within it) increases with enormous rapidity. Müller (1910) remarks that when organisms (e.g. typhoid bacilli) are injected into the peritoneal cavity they at first decrease in number owing to the bactericidal effect of the body fluids but later on increase again. It is during this early stage when the injected organisms are decreasing rapidly that the staphylococcus albus (and possibly, in animals, the bacteria present in the internal organs) are increasing rapidly. A culture removed during this period might well convey the impression that a mutation had occurred.

Dudgeon and Sargent (1907) mention that the staphylococcus albus is often quite non-pathogenic in the peritoneal cavity of the guineapig.

After death there is a rapid invasion of the peritoneal cavity by organisms, particularly by *B. coli* from the gut, so that the true nature of the infection becomes obscured. In illustration of this point, Dudgeon and Sargent (1907) record a case of pneumococcal peritonitis in which the peritoneal exudate one hour after death gave a pure culture of pneumococci, whereas 26 hours later *B. coli* alone could be recognised in the same exudate.

It is not possible, therefore, to exclude in Major Horrocks's experiments the possibility of an invasion of the peritoneal cavity from the gut, following either the inoculation or the death of the animal.

(5) In none of these four experiments was any attempt made to test the new strain by subculture or passage to ascertain whether it would revert. In two experiments the original strain of *B. typhosus* had, within a few hours of its injection, completely disappeared and in none of the experiments were any intermediate forms observed which might be regarded as linking up the new strain with the original one and suggesting a transmutation. All the organisms were apparently of the same type. Moreover the agglutination reactions betrayed no sign, in the only instance in which they were tested, of any connection between the new strain and the original *B. typhosus*. The continuity, therefore, of the two forms cannot be regarded as proved. Moreover if the variants were really derived from the original strain of *B. typhosus* one would have expected them to be present, like the typhoid bacilli, in the blood stream as well as in the peritoneal cavity. One possible explanation is that the change was dependent upon the influence of some agent existing in the peritoneum but absent elsewhere. This will be referred to again (*vide* p. 126).

(6) A repetition of the experiments was made in only two cases (*e* i and *e* iii) and without yielding similar results—indeed the results differed from those first obtained in such a way as to suggest that the original cultures, in both cases, contained strains of bacteria differing widely, at any rate in their virulence.

(7) If the continuity of the two different strains were established in each case, what would be the significance of these changes? The transition from *B. typhosus* to an atypical coliform organism may be regarded as a variation on the part of the typhoid strain, probably temporary in character and of the same nature as those discussed in an earlier part of this work (*vide* p. 11). The transition from *B. typhosus* to a Gram-positive coccus is more difficult to explain but the observations of Adami and others would suggest that, in this case also, a temporary variation and not a true transmutation might have been brought about. Adami (1892) observed that the addition to a medium of substances inimical to the life

of *B. typhosus*—for example, carbolic acid or creosote—made this bacillus in such a medium assume temporarily the form of non-motile cocci or diplococci. Again, Adami, Abbott and Nicholson (1899) obtained from the bile in guineapigs and also from the peritoneal fluid in man, under certain conditions, coccic forms of *B. coli*. These were present as diplococci or short chains of 3 or 4 cocci; they were non-motile, non-fermenting and did not produce indol; their growth on the surface of agar at first closely resembled that of a streptococcus, the colonies were white and opaque. Intraperitoneal inoculation into a guineapig increased their fermenting power and, after 3 passages, yielded normal *B. coli*. They found evidence that *B. typhosus* yielded similar modified coccic forms when acted on by peritoneal and other fluids. They describe coccic forms of *B. typhosus* in the mesenteric and retroperitoneal glands. They mention that in some cases the action of ascitic and peritoneal fluids in this respect is so marked that it was difficult to obtain complete reversion to type. They found, also, that *B. coli* injected into the blood stream in a rabbit appeared in these coccic forms within half an hour in the liver and the bile, though similar forms were not found in the systemic circulation. If the modification in a strain of *B. typhosus* which Major Horrocks describes was due to the same agency, one can understand why the variant was only found in the peritoneal cavity and not in the heart's blood. In his later experiments, however, cocci possessing the characters of *S. faecalis* were obtained not only in the peritoneal cavity but during culture on artificial media. In the account of these experiments, however, there is much to suggest that the original strain was not a pure one.

(*f*) *The change from* B. typhosus *to* B. faecalis alcaligenes *after growth in the diluted and filtered urine of a typhoid carrier and the further changes from* B. faecalis alcaligenes *to* B. coli *on passage.*

(Page 237, exp. I.) The urine of a typhoid carrier "S" was diluted 1 in 10 with tap water and allowed to stand 11 days. It was then filtered through a Pasteur candle (F) without pressure and shown to be sterile by "prolonged incubation"

at 37° C. after plating. The filtrate was then inoculated with a 48 hours' growth of *B. typhosus* isolated from the stool of a carrier "C" and a week later plated out on bile salt neutral red lactose agar. Two or three colonies were examined. One gave the typical reactions of *B. typhosus* but two other colonies failed to agglutinate with typhoid serum and corresponded in their reactions to *B. faecalis alcaligenes*. One week later the latter organism alone was found and it persisted unchanged for several months afterwards.

Criticism. The same criticism applies to this experiment. The original strain was not grown from a single organism. "A particle," we are told, of the growth was added to the solution, sufficient to yield 480 million bacteria to each cubic centimetre. The purity of such a strain cannot be guaranteed. The original culture was derived from a stool and it is said to have yielded an organism closely resembling *B. faecalis alcaligenes*. The experiment was however repeated twice over with a laboratory strain with the same result—a fact which considerably discounts this objection. The new strain might, again, be regarded as a variant of the original *B. typhosus* which, as a result of growth under conditions inimical to its vitality, had suffered a loss of power with respect to its fermenting properties and also its property of agglutinating with typhoid serum. Its other agglutinative characters were not examined, but a similar organism obtained in the same manner from a laboratory strain was found to possess slight power of absorbing agglutinins from antityphoid serum. The new strain was, however, further tested ("culture 33" page 242) by being alternately passed through the peritoneal cavity of a guineapig and subcultured on agar. In one experiment, after the 3rd passage, the peritoneal fluid removed at the end of 6 hours contained *B. coli*, but the fluid removed at the end of 12 hours contained not *B. coli* but the new strain of *B. faecalis alcaligenes* which had been injected.

Only three passages were made—the investigation, that is to say, was not persisted in long enough to decide whether the new strain was capable of reverting or not.

The recovery of *B. coli* after the 3rd passage Major

Horrocks considers may have been due to an invasion by this organism from the gut but was more probably due, in his opinion, to the still further modification of the strain injected, inasmuch as "reversion" apparently took place a few hours later. The disappearance of the *B. coli* might however be explained on other grounds. The strain of *B. faecalis alcaligenes* undergoing passage may have been contaminated with *B. coli* between the second and third passages; the former organism after its two passages might be expected to be more resistant to the body fluids which destroyed the latter.

(*g*) *The change from* B. typhosus *to* B. faecalis alcaligenes *after growth in the diluted and filtered urine of a typhoid carrier and the further change from* B. faecalis alcaligenes *to* Streptococcus faecalis.

(Page 238, exp. II.) The sterile filtrate from the urine of a typhoid carrier "S" was again inoculated with *B. typhosus*—the strain used, on this occasion, being a stock laboratory strain "R" of unimpeachable character. The result was similar to that in the previous experiment. Side by side with colonies of typical *B. typhosus* were found colonies of an organism ("35 A. Col. 2") which corresponded with *B. faecalis alcaligenes* but possessed a slight power of absorbing agglutinins from antityphoid serum (p. 242). Later the latter organism alone was found and it persisted unchanged for many months. The experiment was repeated in exactly the same way and with the same result, *B. faecalis alcaligenes* emerging (p. 240, exp. IV).

These two experiments are simply a repetition of the experiments already discussed, a laboratory strain of *B. typhosus* being used instead of a carrier strain.

When the inoculated filtrate used in the first experiment (p. 238, exp. II) was 6 months old, a loopful of it was added to a broth tube and a 48 hours' growth plated on MacConkey's medium (p. 239). Colonies of *B. faecalis alcaligenes* were again found but with them smaller colonies of a streptococcus closely resembling *S. faecalis*.

The strain of *B. faecalis alcaligenes* obtained in the

first experiment (p. 238, exp. II)—known after this as "35 A"—was further tested (p. 243) by successive passages through the peritoneal cavity of the guineapig. The fluid removed after the 8th passage when subcultured into broth showed short-chained cocci and diplococci but when subcultured on to agar gave, in addition to these cocci, the original bacillus "35A." The latter in broth again yielded the short-chained cocci and these on agar gave a bacillus once more—but this time not "35 A" but a fermenting coliform organism.

The original strain of *B. faecalis alcaligenes* (obtained in Exp. II, p. 238) was a second time tested in the same way (p. 243). It showed no change in character until the 18th passage when it gave rise to a fermenting bacillus of the *B. coli* type which on the 19th passage reverted to *B. faecalis alcaligenes*. This last-named organism, after 7 more passages in one experiment and 8 more passages in another, gave rise to a fermenting *B. coli* type of organism "in pure culture" and this, on planting in broth, yielded *B. faecalis alcaligenes* once more but, with it, cocci corresponding to *S. faecalis*. The latter after 3 passages remained unchanged.

Criticism. The transition from the non-fermenting *B. faecalis alcaligenes* to the fermenting coli type and back again, may be regarded as no more than another example of variation similar to those quoted in an earlier section of this work. Again, in the "passage" experiments, one or other type may have been an invader from the gut, the apparent reversion at a later passage merely representing the destruction of the invader which had not been "hardened," so to speak, by previous passages. Only one guineapig was used for each "passage" experiment in a series of passages. If more than one had been used some check would have existed on possible errors due to this cause. The repeated transitions from *B. faecalis alcaligenes* to *S. faecalis* and vice versa are more difficult to explain, for *S. faecalis* appeared not only after passage but during cultivation also on artificial media. One is almost forced to the conclusion that Major Horrocks was dealing with a mixed strain of the two organisms and that changes in the conditions of growth at one time

fostered the growth of the first organism almost to the exclusion of the second, and at another time fostered the growth of the second almost to the exclusion of the first.

If, after each appearance of *S. faecalis*, the strain had been guaranteed pure by the method of successive plating and growth from a single organism, the results obtained would have had more weight.

It is worth noting that the strains of *B. faecalis alcaligenes* used in all these experiments, and supposed to have been derived from *B. typhosus* in the first place, showed no tendency to revert to *B. typhosus*. It is also interesting to compare these experiments with those of Adami, Abbott and Nicholson (1899) who obtained from *B. coli* grown in peritoneal fluid cocci which did not ferment sugars or form indol, and yielded colonies which were white and opaque and resembled those of a streptococcus. After 3 passages through the guineapig these cocci yield short bacilli which however were still unable to ferment sugars. They state that *B. typhosus* was modified in much the same way under similar conditions.

(*h*) *The change from* B. typhosus *to* B. faecalis alcaligenes *after growth in the diluted and filtered urine of a typhoid carrier.*

(Page 239, exp. III.) This experiment was practically a repetition of the last, the same strain of *B. typhosus* being used (laboratory stock "R") but the urine was that of a different typhoid carrier "I". The result was the same, colonies of typical *B. typhosus* being found at first but after a month's interval colonies of a non-fermenting and non-agglutinating coliform organism being alone found. To this experiment the same criticism applies. 19 other experiments were made but with negative results.

Summary. The evidence that Major Horrocks brings forward in support of the claim that in the course of his experiments transmutation occurred is inconclusive. He is unable in any case to guarantee as pure the culture with which he was dealing. He is unable to exclude definitely, in some of his experiments, the occurrence of a secondary

invasion in the living body. Lastly, even if the evidence established the continuity between his original strains and the new ones he obtained, the changes may possibly have been merely examples of variation no greater in degree than many that have been recorded by other observers. In other words, the strains of atypical *B. coli* and those closely resembling *B. faecalis alcaligenes* or *S. faecalis*, may conceivably have been variants of his original strains of *B. typhosus* whose true identity would have been disclosed by more prolonged efforts to obtain reversion or more thorough investigation with regard to their agglutination reactions.

II. The relationship between members of the enteritis group of bacilli—*B. enteritidis* "Gaertner" and the paratyphoid bacillus of the "Aertryck" or "Flügge" type.

The question of the specific character of these organisms has been much discussed. They are generally recognised as distinct species but evidence has been brought forward from time to time suggesting that they may be transmuted one into the other.

A. *Schmitt's experiments.*

Schmitt (1911), for example, claims that in certain experiments conducted by him strains of the paratyphoid bacillus of the Flügge type became changed within the animal body into the Gaertner type of bacillus. The details of the experiments are briefly as follows.

Experiment I. On July 17th, 21st and 28th he fed a young calf on milk to which had been added (in amounts varying from 1 to 50 c.c.) a broth culture of a Flügge type of organism—without apparent effect, except that the blood serum which previously did not agglutinate the organism now did so in dilutions of 1 in 35.

On August 3rd the same strain of organisms was injected subcutaneously into the calf, with the result that the calf became ill. An organism ("Pgst I") was isolated from the

calf's blood on the same day. It was found to be agglutinated by the animal's blood serum in dilutions of 1 in 50—60 although, again, serum which had been taken from the calf before the experiments began failed to agglutinate it. (A calf serum, immunised against a Gaertner strain from cattle, was on the same day injected intravenously but only made the calf more ill.) A broth culture of this organism, Pgst I, was injected into the same animal on August 7th and again on August 10th, giving rise only to a slight febrile reaction on each occasion.

Experiment II. On August 25th, the strain already mentioned (Pgst I) as having been isolated from the first calf's blood was suspended in normal saline and sprayed into the nose of another calf—without apparent effect. On August 28th a similar suspension was injected into the animal's mouth with the result that the calf became ill. An organism (Pgst II) was isolated from the calf's blood. On September 4th a saline suspension of this organism was injected intravenously into the same calf, which died a few hours later. From the blood, intestines, muscles and bone marrow was obtained an organism Pgst III. The "Schluss-serum" was found to agglutinate the original Flügge type and also the strains Pgst I, II, and III—in dilutions of 1 in 3000—4500.

The great interest of the experiments lies in the observation that these later strains isolated from the blood of the calf were found to correspond in their agglutination reactions, not with the original Flügge type but with the Gaertner type of bacillus—a conclusion confirmed by absorption tests. Schmitt maintained, therefore, that passage through the calf modified the agglutination properties of the human paratyphoid bacillus ("Flügge") so that it came to resemble the calf paratyphoid bacillus ("Gaertner").

Two possible fallacies at once suggest themselves. The type of organism which made its appearance later in the experiment might have been present as a contamination either (*a*) in the original strain or (*b*) in the bodies of the animals inoculated.

(*a*) As regards the first alternative, it is conceivable that

if bacilli of the Gaertner type were present in the original strain, but in such scanty numbers as to escape detection, these few bacilli might in the living tissues multiply with such rapidity as to become ultimately the predominant organism. The precautions taken to secure the purity of the original culture would presumably exclude such a source of error.

(*b*) In the second place, bacilli of the later or Gaertner type might conceivably have been present, before the inoculations were made, in the bodies of the calves themselves. This possibility appears at first sight to be excluded by the fact that the serum of the calf before inoculation failed to agglutinate the organism recovered afterwards, although the serum after inoculation was able to do so. This observation is not, however, final.

Savage (1907–8) and other observers have recorded the presence of *B. Gaertner* in the intestines of healthy young calves. Its presence in the intestines of the calves inoculated in these experiments was not definitely excluded.

The repeated administration in the food of as much as 50 c.c. of a young broth culture of a pathogenic organism would be likely to cause an inflammatory reaction in the bowel and such inflammation might lead, not only to an enormous increase in numbers on the part of any other pathogenic organism present, but also to an exaltation in their virulence. We have referred elsewhere (*vide* p. 80) to such a sequence in the case of *B. coli* in the intestine during an attack of typhoid fever and in inflammatory conditions resulting from improper food.

Such an increase both in numbers and in virulence on the part of the organism we are discussing might well pave the way for its invasion of the system as a whole and lead to its appearance in the blood and internal organs, in a manner analogous to the invasion of the body by saprophytic organisms of heightened virulence in the cavity of an inflamed uterus. At the same time the blood serum would acquire agglutination properties which it did not possess when the bacilli were few in number, of low virulence and restricted to the lumen of the intestine.

B. *The experiments of Mühlens, Dahm and Fürst.*

These writers (1909) have recorded experiments which suggest a similar transmutation. They fed a large number of mice on meat which was thought to have been infected although a preliminary bacteriological examination proved negative. Over 50 per cent. of the mice died.

A bacteriological examination of the faeces of 56 mice was made. In 20 cases none of the paratyphoid group of bacilli were detected. Aertryck's bacillus was found to be present in 24 and Gaertner's bacillus in 13 cases.

These results suggest that one type may have arisen from the other within the animal body.

As in the experiments already discussed, two possibilities have first to be excluded—namely, the possibility of contamination (*a*) in the original strain and (*b*) in the bodies of the mice used for the experiment.

(*a*) All that can be said by way of excluding the first of these alternatives is that the simultaneous presence of both the Aertryck and the Gaertner type of organism in infected meat is contrary to experience and was thought by other writers (Zwich and Weichel, 1910) to be highly improbable in this instance.

(*b*) With regard to the second alternative, no preliminary bacteriological examination of the mice used in the experiment was made. The faeces of 40 control mice were examined and *B. Gaertner* was discovered in one case only. Zwich and Weichel (1910), on the other hand, found that out of 177 healthy mice 28 gave *B. Aertryck* in the faeces.

The bacteriological examination of the 40 control mice with a negative result in 39 cases is, again, open to the criticism that paratyphoid organisms might have been actually present at the time but in such small numbers that they escaped detection. Any such organisms present, in equally small numbers, in the other mice would find a nidus for their growth in the unhealthy and inflamed condition of the intestine which would result from feeding the mice on infected meat, while the disturbed functions of the bowel, by

hastening its evacuation, would bring about the subsequent appearance of these organisms in the faeces.

C. *The author's experiments.*

The following experiments were suggested to the writer by Professor F. A. Bainbridge as likely to throw some further light on this aspect of the question and were carried out at the Lister Institute under his kind supervision.

Experiment I. 24 healthy guineapigs were chosen and 12 of these were confined in 3 cages. On August 13th one of the four guineapigs in each cage was removed and given in its food 1 c.c. of a 48 hours' broth culture of *B. enteritidis Gaertner*. Every precaution was taken to prevent, as far as possible, any external contamination of the guineapigs with the food and they were then returned to their respective cages. This culture of *B. Gaertner* was made from a laboratory strain the agglutination reactions of which had been repeatedly tested and had been found to be constant.

A bacteriological examination of the faeces of the 12 guineapigs was made subsequently on three occasions, namely on August 23rd, September 19th and October 9th—bacilli of the paratyphoid group being identified by agglutination tests. During this period each cage was kept separate from the others and none of the guineapigs were removed from their cages except for the necessary examination on the three dates mentioned. Up to the time of the first examination on August 23rd all the guineapigs remained well, but subsequently three of them died and the remainder exhibited varying degrees of malaise and intestinal disturbance. The following scheme shows the type of organism found in the faeces at each examination.

N.B. Guineapig No. 1 in each cage was given 1 c.c. of a broth culture of *B. enteritidis Gaertner* on August 13th. "0" indicates that neither the Gaertner nor the Aertryck type of organism was isolated.

The faeces of the remaining 12 guineapigs, which had been kept quite apart from the others, were carefully investi-

gated at the date of the first examination (August 23rd). Gaertner's bacillus was not found in any case and Aertryck's bacillus in one case only.

Cage	Guineapig	1st examination August 23rd	2nd examination Sept. 19th	3rd examination Oct. 9th
A	No. 1	Aertryck	Dead	—
	No. 2	0	Aertryck	Gaertner
	No. 3	Gaertner	Dead	—
	No. 4	Gaertner	P. M. Gaetner Aertryck	Gaertner
B	No. 1	Aertryck	0	0
	No. 2	Aertryck	0	0
	No. 3	Gaertner	Dead	—
	No. 4	0	0	(no faeces)
C	No. 1	Gaertner	Aertryck	0
	No. 2	Aertryck	Gaertner	0
	No. 3	Aertryck and Gaertner	(no faeces)	—
	No. 4	0	(no faeces)	—

These experiments appeared to lend further support to the theory that the two types of organisms were capable of transmutation. It is necessary, however, again to emphasise the fact that a negative result in the case of all but one of the animals examined as a control, does not prove the absence of organisms—it only proves their scarcity. A subsequent increase in their numbers might at once have revealed their presence. Such an increase might be apparent only, due to a simple disturbance of the functions of the bowel, such as diarrhoea, which would dislodge the organism from its usual habitat, carry it to a lower part of the bowel and hasten its evacuation. The increase in numbers might, on the other hand, be a real one, brought about by a lowered vitality of the body as a whole and local inflammatory changes. It is to such factors that we attribute the enormous number of *B. coli* found in the stools of patients suffering from cholera.

All these factors were, no doubt, operative in the case of the three guineapigs which were actually fed with the culture of *B. Gaertner* and may explain the subsequent discovery of

B. Aertryck in the faeces of all of them. The remaining guineapigs may have been infected by *B. Aertryck* from the faeces of these, at one stage or another, owing to their food becoming contaminated. The same factors would explain, in their case, the later appearance of *B. Gaertner*.

It is, however, to be noted that at the 1st examination (August 23rd) of the guineapigs in cages A and B, while both those which had been fed with the Gaertner culture were passing *B. Aertryck* in their faeces, three of the six animals which had *not* been fed with the Gaertner culture were passing *B. Gaertner*. To explain this on the grounds that the food of these three had been contaminated by the faeces of the Gaertner fed guineapigs, we must assume that the latter had, at some time previous to the examination, been also passing *B. Gaertner* in their faeces and that this organism had only later given place to *B. Aertryck*.

That the factors we have been discussing do actually lead to the detection in the faeces of organisms which previously did not appear to be present, the writer endeavoured to prove by further experiment.

Experiment II. On August 25th six apparently healthy guineapigs were chosen and labelled Nos. 1 to 6. A fragment of the faeces from each guineapig was shaken up in malachite green broth and after incubation the latter was plated out, two sterile plates being used for each guineapig.

Both plates from No. 3 showed white colonies. A culture from these colonies was grown in broth for 48 hours and then passed through the sugars by which means it was identified as *B. proteus*. In the case of the remainder the five pairs of plates all proved to be sterile.

The guineapigs were then for several days fed on green vegetables and bran soaked in castor oil—a diet designedly unwholesome and calculated to make the animals ill and also to set up some intestinal catarrh. On August 30th a fragment of faeces from each guineapig was again shaken up in malachite green broth and this, after incubation, plated out as before on two sterile plates.

In the case of No. 1 and No. 5 no growth was apparent in

the tubes and both pairs of plates remained sterile. In the other four tubes growth took place with gas formation and the four pairs of plates all showed colonies. Broth cultures were made from these colonies and yielded strains which gave the sugar reactions of *B. proteus*.

The writer failed to demonstrate the presence in any case of organisms of the paratyphoid group. The experiment however was successful in demonstrating that bacteria which failed to give evidence of their presence in the faeces of a healthy guineapig might make their appearance in the faeces of the same animal after it had been given for a few days unwholesome and irritating food.

This conclusion lends weight to the suggestion already made that the results obtained by Schmitt, and also by Mühlens, Dahm and Fürst, might possibly be explained by the presence of a secondary invader.

Their experiments are, in both instances, open to one further criticism. The identification of the paratyphoid organisms was made to depend solely upon their agglutination reactions. If it is admitted that the power to form and to absorb specific agglutinins on the part of an organism is subject to variation it must be recognised that such tests alone are insufficient to establish the identity of the organism. In other words, it is within the bounds of possibility that only one type of organism was actually present, but that its agglutination properties varied.

Such a contingency would be likely to arise in the case of two organisms so closely allied as *B. Aertryck* and *B. Gaertner*. An elaborate investigation into the agglutination properties of these two organisms was conducted by Sobernheim and Seligmann (1910). Pure colonies of numerous strains were secured by the Indian ink method. They found that colonies derived from the same strain and growing side by side differed in their agglutination reactions. The same strain differed at different times. The agglutination reactions, in some instances, became altered after passage through the mouse and after a culture had been heated. In some instances the injection of living bacilli yielded a serum which was much more variable

in its agglutinative powers than a serum obtained by means of a dead culture. Some strains gave doubtful reactions. The power of the same strain to form agglutinins and to bind agglutinins appeared in some cases to differ. They therefore concluded that the agglutination reactions did not constitute a specific test.

We may interpret these results in one of two ways. We may decline to recognise the two types as representing distinct species; or we may continue to regard them as distinct species and acknowledge that their agglutination properties are liable to variation. In either case the experiments quoted are deprived of all significance as examples of transmutation.

CHAPTER X

SUMMARY

It will be evident from the foregoing pages that practically every character of bacteria is liable to vary at different times and under different conditions. These variations are of two kinds, *spontaneous* or "intrinsic"—that is to say due to tendencies inherent in the organism itself—and *impressed* as a result of external influences. These modifying influences have been enumerated (Chapter II) and examples given of the variations they produce.

In many cases an organism may appear to vary although no variation actually takes place, and in other cases what appears to be a "spontaneous" variation is actually an "impressed" variation due to external influences which have not been recognised by the observer. These various sources of error have been enumerated and discussed in Chapter III.

A tendency to vary in a particular way—either spontaneously or in response to external stimuli—may be so characteristic of a certain organism as to be in itself almost specific in character, and so far from confusing its identity may actually make this more apparent. The pleomorphism of *B. diphtheriae*, the tendency of *S. scarlatinae* to assume a bacillary shape, the tendency of *B. paratyphoid B* to form papillae on raffinose agar, will serve as examples.

No single property of bacteria can be regarded as specific nor does the occurrence of variation in respect to any one quality or function, or to several of them simultaneously, necessarily imply a loss of specific character on the part of the organism concerned. This is well illustrated by the morphology of bacteria. A certain appearance may be spoken of as "characteristic." This does not mean that it is invariable but merely that the organism shows a tendency to

present such an appearance rather than another. *B. coli* in the peritoneal cavity in the case of ascites may take the form of a diplococcus; in milk or in urine it may develop into a dense network of branching filaments resembling *B. anthracis*, but these changes in form do not imply any obliteration of the specific character of the organism itself.

We have already referred in this connection (*vide* p. 38) to the analogy of a regiment of soldiers at manœuvres and a mass meeting of miners at the pithead. The various military formations assumed by the first are as characteristic as the concentrically arranged crowd formed by the second—so much so that an observer at a distance might from the appearance of these "zoogleic forms" state with confidence the character of the units composing them although too far away to identify the latter. A crowd of pitmen on strike might, however, march in military formation and a regiment of soldiers at a boxing match take the form of a crowd concentrically arranged—each reproducing, that is to say, the appearance regarded as typical of the other. This would not indicate that the pitmen were changing into soldiers or the soldiers into pitmen. It is true, nevertheless, that the arrangement most frequently observed in one or the other case does indicate a tendency on the part of the individual unit and may, therefore, afford a clue to its identification. The behaviour of a civilian under certain circumstances may furnish evidence of a military training and deserters from the colours are not infrequently recognised by such means. In a similar way, the occasional assumption by the bacillus of diphtheria of clubbed and branched forms, while helping us to identify it, also provides us with a clue to its mycelial ancestry (Kanthack and Andrewes, 1905).

Many of the variations exhibited by bacteria do in fact, represent steps in the evolutionary process by which, in the past, they have become differentiated—the individual organisms living over again, as it were, the life history of the race. This would appear to be the explanation of many variations in morphology (Chapter IV). Others again represent the advance along new lines of this same evolutionary process, leading to further specialisation and differentiation.

This aspect of the subject has been considered at length in the sections dealing with Fermenting Power and Virulence (Chapters V and VI).

Since we have no absolute criterion as to what constitutes a "species" amongst bacteria, dissimilarity in the several characters they present is our sole guide to classification. In other words the distinction between a "variety" and a "species" depends simply on less or greater divergence in character. The difference, therefore, between variation and transmutation is one of degree only; or, looking at the matter from another standpoint, we may say that the *same* degree of deviation from type may be interpreted in one case as variation and in another as transmutation. This will be readily understood if it be borne in mind that the various types or "species" of bacteria which we are able to distinguish have developed from a common stock. In the case of some of them the differentiation dates from a remote past and the specific characters are comparatively fixed. In the case of others differentiation is of more recent date and the newly acquired characters are less permanent and "reversion" in one or other character is more frequent. In yet a third class—the groups of closely allied organisms—the gradual process of differentiation is only now taking place and it is not yet clear which characters are of specific value. During the process of evolution, in all its stages, there is a tendency shown on the part of the organism to revert towards the original type. Such reversion in one or more characters, although of no greater significance in the case of one class or another of the three we have described, is likely to be differently interpreted. If differentiation is well advanced, a partial reversion in character will merely present itself as an unimportant variation. If differentiation has not progressed very far, a reversion no greater in degree may confuse the identity of the organism concerned sufficiently to suggest the possibility that transmutation has occurred. If the process of differentiation is still incomplete, a reversion in character even smaller in degree may entirely obliterate the faint lines of division that we have been able to trace out. The error of assuming too hastily that

transmutation has occurred will be prevented by a proper consideration of, firstly, the biological characters of the organism in question *as a whole* and, secondly, the question of the *stability* of the characters which distinguish it.

With regard to the first of these questions, we have shown in the sections dealing with Morphology, Fermenting power, Virulence and Pathogenesis (Chapters IV–VII) the danger of relying upon any one of these characters alone for the purpose of identification or of classification.

In the case of widely divergent types a single character may sometimes suffice to distinguish one organism from another but even in such a case, if that character is liable under any circumstances to variation, it obviously cannot be trusted as an infallible guide.

The pathologist is in the same boat, in this respect, with the ethnologist. Certain "race groups," e.g. the Teutonic, the Mongolian, and the Negroid, though conceivably derived from a common anthropoid stock, are sufficiently differentiated to be readily distinguished by a single character. For example, the flaxen hair of the German, the matted black hair of the Negro and the straight black hair of the Jap are sufficiently characteristic of their respective race-groups. Such a distinction, however, breaks down between the races within the groups themselves and other characters must then be considered in addition. In some cases, again, the process of differentiation is still incomplete, individuals approximating now to one and now to another recognised type, and a consideration of *all* the characters may still leave the observer in doubt as to the correct classification.

The ethnologist has learnt, moreover, that certain characteristics are not to be regarded as racial in character. For example—to return to our previous illustration—the pigtail of the Chinaman and the shaven poll of the Thibetan priest, the flowing locks of an Italian impressario and the tonsured crown of a Romish monk, are not racial characters at all but artificial modifications. They do, however, signify a certain environment and training and this is precisely the case with many of the variations which the pathologist meets with

amongst bacteria. In other words, a study of such variations in a given case may afford valuable and trustworthy information as to the source from which the particular strain of organisms has been derived.

This subject would repay further investigation. One or two instances may be given here to demonstrate its importance.

Rosenow (1912–13) found that the ordinary streptococcus pyogenes, if grown in unheated milk, became modified in its morphology, its cultural properties and its virulence. He had previously isolated from several cases of epidemic sore throat a streptococcus which possessed precisely similar modifications in character. The epidemic had been recognised as "milk-borne" but, had its origin been in doubt, the unusual characters of the organism concerned would obviously have provided a clue.

Ohlmacher (1902) isolated branching filamentous forms of *B. coli* from the heart's blood in a case of septicaemia. He quotes various observations to the effect that residence in the biliary passages develops this unusual morphology in *B. coli*, and he therefore considers that the original source of the systemic infection in this case was in the region of the gall bladder or bile ducts.

Moreover the degree to which the modifications persist on subculture is a measure of the time during which the organism was subjected to the modifying influence. This brings one to the second question, the *stability of the variations* produced.

Remarkable differences are to be observed in the degree of permanence exhibited by a variation in different cases and it is difficult to decide upon what factors these differences depend.

We have already referred to the fact that variations may be either "spontaneous" in character or "impressed" upon the organism by external agencies. *Spontaneous* variations may be of several kinds and the nature of the variation may itself decide its degree of permanence.

1. Some variations represent an early stage in the *life history* or are due to imperfect development, and are seen

in young or backward cultures. We have spoken of the atypical morphology of a young culture of the Klebs-Loeffler bacillus, which renders it difficult to distinguish it from Hofmann's bacillus (*vide* p. 42), and of its inability to ferment glycerin and lactose (*vide* p. 55). Such differences are comparable to the juvenile features and unskilled hands of a class of schoolboys and tend to disappear of their own accord as the strain grows and develops.

2. Other variations represent *senile* changes or are due to lowered vitality, and are seen in old or worn out strains. The loss of motility, or of pigment production, in an old culture will serve as an example. Such variations are comparable to the slow steps and grey hairs that characterise a party of old men and will tend to become more and more developed unless some external influence intervenes and, by effecting a radical change in the conditions of growth, contrives to rejuvenate the strain.

3. Others again are degenerative in character or are due to *atavistic* tendencies—such as, for example, the appearance of branched and clubbed forms of the tubercle bacillus. These variations are comparable to some forms of mental impairment in a family, or to defects such as harelip. They may be passed on from father to son and so persist, or they may disappear, but in the latter case they tend to recur in a later generation.

4. Others, finally, are *evolutionary* in character and represent a higher specialisation on the part of the organism—such as, for instance, the development by *B. typhosus*, after a long training, of power to ferment lactose, or the acquisition on the part of a feebly pathogenic organism of the quality of extreme virulence. Such changes are analogous to the development of a national genius for literature or conquest. The more highly specialised a function is the more easily does it become deranged and a character, therefore, of this kind, is readily lost. For example, however permanent other newly acquired characters in bacteria may appear to be, variation in the direction of increased virulence seldom is so and almost invariably proves unstable.

It is easy to see how, in every one of the four classes we

have mentioned, two or more variations may be constantly associated. In some cases the association is explained by the fact that both variations are due to lowered vitality. For example, the loss of power to produce pigment may be associated with the loss of power to liquefy gelatin or to grow on certain not very favourable media—all these functions being dependent upon the vitality of the organism.

Again, the evolution or higher specialisation of an organism may involve simultaneous modification in two or more directions. These modifications may all represent a casting off of saprophytic characters by the organism in question on its entry upon a parasitic career. For example, a saprophyte may derive its vital energy from the sunlight by means of a pigment, comparable to the chlorophyll of a vegetable cell, or from carbohydrate food through its ability to ferment it. When it becomes parasitic, and in many cases pathogenic, it is cut off from sunlight and must subsist on the body fluids. We may find therefore that the acquirement of virulence is associated with the loss of power to form pigment and to ferment sugars.

In the same way, the constant association between two different variations may be due to the fact that the young strains which show them have not developed their adult powers, or to the fact that the variations are both signs of degeneracy or atavism.

"*Impressed*" variations show even greater differences in their degree of permanence. In some cases a variation is only maintained while the influence which caused it continues to act. In others the variation persists for a shorter or longer period after that influence is withdrawn. In others again the variation is apparently permanent and persists under normal conditions of growth indefinitely. We use the expression "apparently permanent" for it is impossible in any case to guarantee the permanence of the characters exhibited by a strain of bacteria. This has been shown both by observation and by experiment. Mention has been made elsewhere (*vide* p. 14) of a strain of bacteria which after nine years' cultivation lost its power to ferment maltose, and of another strain which after five years cultivation lost its power to produce pigment.

Twort found that *B. typhosus* grown in a lactose medium retained its character as a non-fermenter of lactose for two years before variation occurred. Eyre and Washbourn found that to raise a particular strain of an avirulent saprophytic pneumococcus to full virulence by animal passage, no less than fifty-three successive inoculations were required. Characters which persisted for periods of two, five and nine years, and withstood a series of over fifty passages through an animal body, might well have been regarded as "permanent." They were, however, only "apparently" so.

Certain principles which govern the stability of impressed variations can, however, be discerned.

1. *The variation may affect all the members of a strain or only certain of them.* In the latter case an apparent reversion is obviously more likely to occur. The rapidity with which this apparent reversion takes place will depend upon the comparative rate of growth of the unaltered organisms and the variants. If the new character is of advantage to the organism it will enable the variants to multiply more quickly and they will gradually get the upper hand. Apparent reversion will not take place as long as the new character continues to confer an advantage upon its possessors but when this ceases to be the case the organisms possessing the new character may disappear and reversion to the original type appear to take place. For example, the acquirement of virulence by some members of a non-virulent or feebly-virulent strain, when this is injected into the living body, gives these variants an advantage as long as they are in the body. If the mixed strain is grown on artificial media the advantage is done away with and the unaltered bacteria, other things being equal, have now as good a chance as the variants of increasing their numbers and the variants may disappear.

We have used the expression "apparent reversion" for it is evident that, unless every member of a strain acquires the new character, the loss of that new character by the strain may be brought about by the dying out of the variants without a single organism having actually "reverted." This fallacy can be readily excluded if care be taken at each step to ensure that

the strain of bacteria under observation is a pure one—that is to say, derived from a single organism by the methods suggested by Barber and others.

2. *The more readily a new character is "impressed" on an organism the longer it is retained*—conversely, the more slowly and reluctantly an organism takes on a new character the more easily is that character lost. The behaviour of *B. typhosus* is a good illustration. This organism can be trained to ferment dulcite in a few days and will then retain the power for many weeks in the absence of that sugar. It cannot be trained to ferment lactose in less than two years and then loses the power in a few days if the lactose is withdrawn.

It must be understood that we are here speaking of "impressed" variations. In the case of "spontaneous" variations the reverse holds true. Bacteria which vary spontaneously with great readiness often revert with equal facility, while those that are tenacious of their normal characters often prove tenacious of any new character they may spontaneously develop.

3. *The longer an organism which has undergone a variation continues to be exposed to the influence which caused it, the longer will the variation persist after that influence has been withdrawn.*

For example, Rosenow (1912–13) isolated a streptococcus, from a number of cases of general infection, possessing unusual morphological and cultural characters which, however, showed reversion on cultivation outside the body. He found that strains isolated from the peritoneal exudate and blood at a later stage in the disease showed these modifications in character to a greater degree than those isolated earlier in the attack.

The behaviour of *B. typhosus* again illustrates this point. If a strain is grown on dulcite medium it acquires, in a few days, the power of fermenting that sugar and this power is retained for some weeks after the strain has been removed from the dulcite medium. Reversion then occurs and the power is lost. If however the strain is grown on a dulcite medium continuously for three months the power to ferment

dulcite is found to persist afterwards, on ordinary media, "permanently."

This observation, based on the results of laboratory experiments, provides a clue, as Adami observes, to the nature of the process by which new races of bacteria are developed. In the laboratory organisms can be exposed to certain modifying influences for many months or even years and the new characters developed by such means are found to persist for long periods before reversion takes place. In nature agencies which possess the power of modifying the characters of bacteria may exert their influence for an indefinite period and the process of reversion in this case may be indefinitely postponed. In other words the new characters developed may appear to be permanent. A variant, however, may retain its new characters indefinitely and show no tendency whatever to revert under ordinary conditions of growth and yet it may still be capable of reverting immediately under suitable conditions. Examples of this are common in the laboratory and may be found in nature. Laurent describes a decolourised strain of *B. ruber* which was grown for 12 months at a temperature of 25–35° C., being subcultured 32 times in this period, without once showing any trace of pigment. On lowering the temperature to 18° C. pigmentation at once reappeared. Again, the diphtheria bacillus is far removed from its mycelial ancestry but under suitable conditions will still display a partial reversion to a mycelial structure. We do not on this account deny the title of "species" to the diphtheria bacillus, for we recognise that the idea of absolute permanence in character is not essential to our conception of a species in the case of bacteria. It is not permanence in character but the degree of resistance to alteration in character displayed by an organism that determines our opinion of its specific nature. In spite of the many minor variations they display there is exhibited by most species of bacteria a resistance to modification—a "vis inertia"—which constitutes true racial stability.

We have seen, then, that the difference between *variation* and *transmutation* is one of degree alone. It is a question of the extent of the modification and the degree of permanence

it exhibits. It is no less true that the process of *transmutation* only differs in degree from the process of *evolution*. Here it is a question of the rapidity of the change.

Let us take by way of illustration the case of a family of ancient lineage, the members of which hold high office in the State and are remarkable for their wealth and erudition. Such a family may have sprung 500 years ago from humble origin and, while the fortunes of one branch have steadily prospered and successive generations have gradually acquired fame and amassed wealth, the original yeoman stock from which it sprang has continued to be represented throughout the centuries, in some corner of the kingdom, by men chiefly remarkable for their deficiency in the riches and learning and reputation for which the others are distinguished. It is conceivable that a son of the older and less distinguished branch of the family, seizing a favourable opportunity, might, by the exercise of the same faculties of industry and thrift displayed by the others, raise himself in the space of a single life-time to a position of wealth and power equal to theirs. We can trace the steps by which, in the course of time, a virulent and highly specialised race of bacteria has been evolved from a less virulent and less highly organised race. We find the two races living still side by side. The question arises whether it is possible under unusually favourable conditions for the process of adaptation and specialisation to take place with such rapidity as to suggest a sudden transmutation.

The conversion of the saprophytic pneumococcus into the parasitic pneumococcus by Eyre, Leatham and Washburn (*vide* p. 115) appears to offer an example. These observers describe the virulent parasitic pneumococcus as requiring for its growth a certain reaction and temperature and particular media (blood agar); it would not grow if the reaction were even faintly acid or at a temperature much below 37° C. and rapidly died out on agar or in broth. It would not liquefy gelatin and in broth formed a dust-like deposit. The avirulent saprophytic variety, on the other hand, grew luxuriantly at temperatures ranging from 37° to 20° C., on agar, gelatin, potato or in broth, whether acid or alkaline, slowly liquefying

gelatin, producing a uniform turbidity in broth, and it retained its vitality for many months; it also exhibited differences in its morphology, "instead of isolated diplococci and streptococci, large masses of cocci and diplococci were found, and forms dividing into tetrads were common." Nevertheless this avirulent saprophytic pneumococcus could, by a single "passage" through a rabbit, be converted into a typical parasitic pneumococcus of high virulence. The occurrence of such a remarkable transition would be regarded as more significant if it were not that both organisms bear the same name and are considered—in spite of the many differences existing between them—to be variants of each other.

If we consider the possibility of a similar transition in the case of two races of bacteria less closely associated with each other, we find little direct evidence in proof of its occurrence —and this often of doubtful value—but a great deal of circumstantial evidence in favour of the supposition that it may occur. We have discussed at length (Chapter VIII) such a possibility in the case of organisms found in close association in the body, such as Hofmann's bacillus and the Klebs-Loeffler bacillus, *Staphylococcus epidermidis* and *Staphylococcus pyogenes*, *Micrococcus catarrhalis* and the meningococcus, and others.

Finally, we have discussed in detail (Chapter IX) the records of certain experiments in the course of which bacteria became so changed in character as to suggest that they had undergone transmutation.

In the first series of experiments—those of Major Horrocks—the results seem to be capable of explanation on other grounds. In the first place adequate precautions do not appear to have been taken to guarantee the purity of the strains at different stages of the experiment. In the second place, many of the changes in character stated to have been observed may be regarded as examples of temporary variation only, similar to those recorded by many other observers.

The second series of experiments—those of Schmitt, and of Mühlens, Dahm and Fürst, and of the writer—which suggest the occurrence of transmutation between different

members of the paratyphoid group of bacilli, are open to the same criticism. In the first place, a temporary variation in one character alone—namely in agglutination properties—would sufficiently explain the results obtained. In the second place, these results may have been due to a secondary invasion—in other words, it is conceivable that there may have been a pre-existing but unrecognised infection in the animals utilised for the experiments. This hypothesis we have shown, from the records of other investigators and by analogy with other processes of infection, to be not improbable; while the writer's experiments further demonstrate the ease with which such a secondary invasion may be overlooked.

In none of these experiments, therefore, can the occurrence of transmutation be regarded as proved, nor, on close examination, does its occurrence appear probable.

A theory which we propose to discuss in conclusion suggests a via media by means of which organisms might conceivably exchange many of their characters and functions without themselves undergoing transmutation. This is the Enzyme theory of disease.

CHAPTER XI

THE ENZYME THEORY OF DISEASE

It is impossible to leave this subject without some further mention of a theory, to which passing reference has already been made more than once in the foregoing pages, namely the Enzyme theory of disease.

This theory predicates that the results which follow, and are regarded as characteristic of, infection by a certain organism —including both the pathological lesions produced and the train of symptoms observed clinically—are caused not only (if at all) by the activities of the micro-organism itself, but by the activities of ultra microscopic bodies of the nature of enzymes which are associated in each case with a particular bacterial cell in the same way that the ferments of yeast are associated with a particular vegetable cell.

If the scattered references to this theory in the foregoing pages be collected together they will be found to constitute a by no means negligible weight of evidence in favour of it. The considerations which lend support to the theory are the following.

1. In the first place, there is the observation that a saprophytic organism incapable at one time of giving rise to disease, even after it has invaded the living tissues, may suddenly acquire pathogenic powers and give rise in the living body to definite lesions and a definite group of symptoms. Harmless organisms such as the saprophytic pneumococcus, the Micrococcus catarrhalis and *B. coli*, for example, may mysteriously acquire the power to produce respectively pneumonia, meningitis and enteric fever. On the other hand, virulent pathogenic organisms such as the Klebs-Loeffler bacillus, the meningococcus and *B. typhosus* may as mysteriously become deprived of their power to produce respectively diphtheria, meningitis

and typhoid fever. Eyre and Washbourn (1899) showed in the case of the pneumococcus that such an alteration in character could be brought about, in one direction, by a single passage through an animal and the reverse change with almost equal facility. We have no explanation of the processes upon which such changes in character depend but we know that many of the conditions which bring them about are precisely those which foster or destroy other properties in organisms which we believe to depend on ferment action (*vide infra*).

2. In the second place, there is the observation that the pathological lesions and clinical symptoms resulting from, and characteristic of, infection by a certain organism may be faithfully reproduced as a result of infection by a totally different organism. For example, we have noted (*vide* pp. 99 *et seq.*) some of the lesions and symptoms of diphtheria to be caused by the pneumococcus, those of scarlet fever and of influenza by *M. catarrhalis*, those of cerebrospinal fever by the Klebs-Loeffler bacillus, by *M. catarrhalis* and by *B. typhosus*, and those of rabies by the Klebs-Loeffler bacillus.

The description of the last example given—a case of rabies due to infection by the bacillus of diphtheria—will bear repetition. It was recorded by Head and Wilson (1899). The diagnosis of rabies was founded on the history and clinical symptoms. "The well authenticated history of a bite on the cheek by an animal, the two months' incubation period, the onset with extreme pain and numbness in the region of the scar, the development of the characteristic laryngeal and respiratory spasms on attempting to take liquids, the spasm at first being slight but later more pronounced and towards the close again feeble or absent, the insomnia, the absence in the beginning of fever which later in the illness became pronounced, the rapid pulse at all stages, the attacks of violent delirium interspersed with periods of calm and complete rationality, the absence of all symptoms pointing towards any other simulating disease and the fatal termination—all serve to make an almost complete picture of rabies." The Klebs-Loeffler bacillus was isolated from the ventricular fluid and detected in the nerve cells of

the medulla. The recognition of this organism was complete and beyond doubt. "Not less suggestive of rabies than the clinical history were the results of subdural inoculations in rabbits with emulsions prepared from the medulla of the patient. There occurred the long period of incubation (20 and 21 days) followed by phenomena similar to those in experimental rabies of rabbits, and other rabbits inoculated subdurally with the medulla of the first rabbits behaved in a similar manner." *B. diphtheriae* was demonstrated after death in the medulla of the rabbits. By a thorough investigation, full details of which are given, infection by the virus of rabies was definitely excluded.

Such phenomena become intelligible on the supposition that both the lesions and the symptoms of a disease result from the activity of particular enzymes which are usually associated with one particular organism but are capable of being associated, under certain conditions, with an altogether different organism.

3. In the third place, representatives of one specific organism, morphologically and culturally indistinguishable from one another, may give rise in the living body to entirely different lesions and symptoms. Indeed, the contrast between the train of lesions and symptoms produced in one case and that produced in another may be as marked as the contrast between the lesions and symptoms produced by two organisms representing two distinct species.

For example, different epidemics of the same disease may present altogether different features. Thus, strains of *B. influenzae*, morphologically and culturally indistinguishable from one another, may give rise to epidemics of "influenza" characterised by symptoms resembling in one epidemic a simple coryza, in another epidemic rheumatic fever, in a third typhoid fever, and in a fourth cerebrospinal meningitis.

Not only do different epidemics present different types of disease but individual cases occurring in the course of one and the same epidemic, and undoubtedly due to infection by the same organism, may exhibit a totally different train of symptoms. We have mentioned elsewhere, the account given

by Andrewes and Horder (1906) of a number of cases of contagious disease, obviously passed on from one patient to another, of which some presented the symptoms of scarlet fever and others those of puerperal fever (*vide* p. 98).

Another remarkable instance (recorded by Dunn and Gordon, 1905) has been already alluded to but is of sufficient interest, in this connection, to warrant a second description. They mention an epidemic in Hertfordshire characterised by an extraordinary diversity of symptoms in different patients. In some cases there were sneezing, coryza and the ordinary symptoms of a common cold. In other cases patients complained of aches and pains all over and stiff neck, and suffered subsequently from great debility; such cases had all the appearance of influenza. In others, again, the illness closely resembled scarlet fever; it began with sore throat, rigors, vomiting, headache, fever and rapid pulse, and was accompanied by a punctate rash at the end of the first 24 hours (followed later by desquamation), the "strawberry" tongue, circum-oral pallor, enlarged cervical glands which in some cases suppurated, and in some patients by complications such as nephritis, arthritis and otorrhoea. A fourth type resembled diphtheria and exhibited a suspicious membrane on the tonsil. A fifth type was notified in some cases as typhoid fever and was characterised by epistaxis, melaena, prostration and, in some cases, it is stated, a positive Widal reaction. Finally, a number of cases, particularly amongst children, resembled cerebrospinal fever and were so diagnosed; these were characterised by profuse nasal discharge, pain in the back of the neck, headache, photophobia and irritability, dilatation of one or both pupils, persistent vomiting, drowsiness, head retraction, paralysis, coma, and sometimes convulsions and death.

Sometimes these widely divergent types were exhibited by the different members of a single family or household struck down by the disease, either simultaneously or consecutively. After a thorough investigation, these observers were convinced that the outbreak of these various types of illness was due to the prevalence and spread of only *one* disease and not a

number of different diseases, and a bacteriological examination of a large number of cases by Gordon showed that the disease was due to infection by an organism closely resembling, if not identical with, *M. catarrhalis*.

Such contrasting groups of symptoms inevitably suggest to our minds that something beside the mere presence of the organism is responsible for them.

4. In the fourth place, one can trace a remarkable resemblance between the conditions which influence the development and the loss of pathogenic power on the part of micro-organisms and the conditions which influence the development and the loss of their power to ferment carbohydrates.

(*a*) The addition, in small quantities, of *an antiseptic*—such as carbolic acid—to the culture medium deprives organisms growing in it of virulence (*vide* p. 75). The same agency will destroy the power of organisms to ferment carbohydrates (*vide* p. 55).

(*b*) The influence of *oxygen*. Pasteur, 30 years ago, found that the virulence of the organism of chicken cholera was better maintained in the absence of oxygen. Anaerobic growth similarly increases the virulence of the cholera spirillum (Hueppe, quoted Adami, 1892). On the other hand, *B. diphtheriae* and other organisms become less toxic if deprived of oxygen. The same factor influences the activity of ferments. In some cases the absence of oxygen inhibits their functions, in other cases it appears to augment them. This is exemplified by the sugar splitting ferments associated with bacteria. For example, anaerobic growth may increase the power of the dysentery bacillus to ferment maltose (Torrey, 1905). Andrewes and Horder (1906) mention a strain of streptococcus which failed to ferment lactose under ordinary conditions but did so readily when deprived of oxygen.

(*c*) *Changes in temperature*. It is characteristic of enzymes that each one has an optimum temperature at which its activities are most effective and also higher and lower limits of temperature beyond which its activities altogether cease. The digestive enzymes in man act most rapidly at the

temperature of the human body—those of cold blooded animals at much lower temperatures. The diastatic ferment of germ barley is most effective at 60° C.—a temperature at which most enzymes are destroyed. The phosphorescence sometimes observed in sea-water is produced by the Micrococcus phosphorescens through the agency of an enzyme the optimum temperature of which is that of the sea.

The enzymes which are associated with bacteria and bring about the fermentation of carbohydrates show a similar behaviour. We find that a strain of bacteria which will ferment a certain "sugar" at one temperature will not do so at another. For example, Wilson (1910) describes a strain of *B. typhosus* which at 22° C. would ferment lactose within two days but at 37° C. failed to do so in a month. Coplans (1909) observed certain strains of *B. coli* which exhibited the reverse phenomenon, fermenting dulcite more readily at 37° C. than at 20° C.

The property of virulence in pathogenic bacteria is likewise governed by temperature. Organisms which are virulent when growing at one temperature lose their virulence when grown at another. For example, *B. diphtheriae*, *B. tetani*, *B. anthracis* and many others (*vide* p. 74) lose their virulence at temperatures much above that of the body. The fact that no bacterial disease in cold blooded animals is communicable to man may possibly be explained on such grounds.

Furthermore, just as the enzymes which ferment carbohydrates are destroyed at temperatures much above 60° C., so we find the property of virulence may be completely removed by subjecting an organism to high temperatures, even though the organism itself survives. The tetanus bacillus is deprived altogether of toxicity by growth at 65° C. for one hour (Muir and Ritchie).

(*d*) Exposure to *sunlight* is another factor which influences both the fermenting power and the virulence of organisms.

(*e*) Finally *symbiosis* is not without influence. Diseased conditions may result from a mixed infection which neither of the organisms concerned is capable of producing alone. It is said that a dog will not succumb to the infection of tetanus

unless it is infected simultaneously with pyogenic cocci. In the same way, processes of fermentation may be brought about by two different organisms growing together in a certain medium which neither can accomplish by itself. For example, neither *B. coli* nor *B. dentrificans* alone can reduce nitrates but if allowed to act upon sodium nitrate together they bring about the escape of free nitrogen.

5. There is, furthermore, a remarkable correspondence between the acquirement of virulence by "animal passage" and the acquisition of fresh fermenting properties by prolonged growth in a medium containing a particular sugar:

(*a*) The virulence acquired by "passage" through a certain animal applies to that particular species of animal; virulence towards another species may be increased at the same time but towards a third species it may actually be diminished. The fresh fermenting power resulting from prolonged growth in a sugar concerns that particular sugar; the capacity of the organism to ferment another sugar may be increased simultaneously while in respect to a third sugar the fermenting power may be diminished.

(*b*) The method of "passage" is more effective in conferring virulence if repeated inoculations are made through a series of animals at short intervals. The prolonged growth in a particular sugar is more successful in developing fermenting power if repeated subcultures are made at frequent intervals on to media containing the sugar.

(*c*) If virulence is readily acquired on "passage" it is easily maintained and is found to persist for a long time on artificial media; on the other hand if it is very slowly developed by "passage" it is quickly lost outside the body. It is so, also, as regards fermenting power. In cases where the property is rapidly developed, by growth on a particular sugar, it is retained for long periods on ordinary media; on the other hand, where it is very slowly acquired it is found that a return to ordinary media is soon followed by reversion in character.

(*d*) Where virulence has been lost only for a short time by a strain of organisms it is quickly restored by "passage";

the power to ferment a particular sugar, if it has only recently failed, is rapidly regained in the presence of that sugar.

6. Bacterial toxins, again, are considered to be of two kinds—extra-cellular toxins, secreted by the bacterial cell into the surrounding medium, and intra-cellular toxins elaborated within the body of the cell and liberated only when the cell itself is disintegrated. The same may be said of the enzymes which ferment carbohydrates. The ferment of yeast, "invertin," which transforms cane sugar into dextrose and levulose, can be separated from the yeast cell. The breaking up of the dextrose into alcohol and other products is a property of the yeast cell itself and the ferment responsible for this second stage can only be extracted when the actual cell body is expressed (S. Martin, 1904). The ferments of the alimentary canal may be distinguished from each other in the same way. One stage in digestion is brought about in the lumen of the intestine by extra-cellular ferments present in the secretions. Another stage is effected within the actual cells of the intestinal wall by intra-cellular ferments acting upon the foodstuffs as they are absorbed.

An emulsion of even a small portion of a glandular organ may possess far more power than its actual secretion, for the former contains the intra-cellular as well as the extra-cellular enzymes. An emulsion of pathogenic bacteria is likewise far more potent than a culture containing the same number of organisms. For example, the smallest fatal dose to a bovine animal of a culture of tubercle bacilli contains 20,000 million organisms. The smallest fatal dose of an emulsion of the bacilli contains only 5000 (Report of English Tuberculosis Commission).

7. In the seventh place, it may be observed that the property of virulence is in many instances associated with the power of producing fermentation. If we study two closely allied organisms, one of them virulent and the other non-virulent, the former will often be found to be the sugar fermenter while the latter has no action in this respect. For example, the Micrococcus catarrhalis is comparatively non-virulent and ferments no sugars; the gonococcus and

meningococcus are virulent and ferment sugars. Again, Hofmann's bacillus is non-virulent and non-fermenting while the Klebs-Loeffler bacillus is virulent and ferments. *B. coli communis* is a sugar fermenter and readily acquires virulence. We may explain the association between the two properties on the ground that both are examples of adaptation and that an organism which possesses unusual power of adaptability in one particular direction may be expected to show a similar power of adaptability in another direction; but the association between virulence and fermenting power lends some support to the supposition that the former may depend upon a process which we have every reason to believe is responsible for the latter, namely ferment action.

8. Bacterial invasion is met, on the part of the body, by measures calculated to destroy the organisms and to counteract their toxins. These measures consist in the elaboration, by the fixed cells of the body as well as by the leucocytes, of various enzymes (Osler and McCrae). The class of weapon forged by the tissue cells for purposes of defence might, perhaps, be thought to give some indication as to the class of weapon it is designed to meet.

9. Many other functions of bacteria, besides the fermentation of carbohydrates, are attributed to ferment action; for example, the formation of indol, the coagulation of milk (Savage, 1910), the liquefaction of gelatin, the production of pigment (Adami) and the development of agglutinins (Duclaux). Moreover these other functions of bacteria, like their power of fermenting carbohydrates, appear to be governed in many instances by the same conditions which we have already mentioned as influencing their virulence. Thus, the presence or absence of oxygen, high and low temperatures, exposure to and protection from sunlight, the presence of antiseptics, are all conditions which markedly affect the production of pigment by bacteria (Adami, 1892).

10. Many of these ferments are separable from the bacteria with which they are associated. Twenty-five years ago it was proved (Bitter, 1887, quoted Wood) that the liquefaction of gelatin by bacteria was due to a ferment which

was independent of the bacteria and survived when the latter were killed by subjection to a temperature of 60°C. Sortinin (*ibid.*) showed that a culture fluid after it had been passed through a Chamberland filter, which removed all the bacteria, still retained the power to liquefy gelatin. Brunton and McFadyean (1889) found that the gelatin liquefying ferment could be isolated by suitable solvents, in the same way that the inverting ferment of yeast can be extracted with ether.

11. Instances may be cited of chemical processes, taking place in the body fluids, which are invariably associated with the presence of certain micro-organisms but which nevertheless have been proved to be brought about by the activity not of the organisms in question but of ferments associated with these organisms and yet separable from them. Such an instance is to be found in the action of the micrococcus ureae. In the presence of this organism the urea of the urine is split up with the formation of ammonium carbonate. In the absence of this organism the process does not take place and if the process has begun the removal of the organism at once stops it. An ethereal extract, however, of the micrococcus ureae has the power of accomplishing all that the presence of the organism itself can effect in this direction. In other words, the results brought about by its presence are due not to its own activities but to those of a ferment "urase" which is in some way associated with it but which can be dissociated from it without any loss of function.

12. If it were possible to discover a parallel instance of dissociation, not between an organism and its *chemical* functions but between an organism and its *pathological* functions, the discovery would give great weight to the theory we are discussing. Now such a parallel can actually be traced in the action of the pneumococcus. Rosenow (1912–13) has recently shown that the artificial injection into the body of the toxins manufactured by the pneumococcus may bring about the death of an animal in one of two ways. It may produce an acute bronchial spasm which proves fatal in a few hours. If the dose of the toxin is small the bronchial spasm may not

be sufficiently acute to cause death and other symptoms and lesions follow which result in the death of the animal in a few days. He discovered that the particular constituent of the toxin responsible for this bronchial spasm could be removed altogether from a suspension of the organism by the addition of blood charcoal, so that the subsequent injection of the filtered fluid failed to cause the bronchial spasm, although it still produced the other symptoms and lesions and led to a fatal termination in a few days. He, likewise, discovered that the same constituent of the toxin could—like the sugar-splitting ferment of the yeast cell and the urea-splitting ferment of *M. ureae*—be extracted with ether and, further, that if a normal saline solution, to which this ethereal extract had been added, were injected into an animal, the typical bronchial spasm was developed in the complete absence of the organism itself.

The force of this analogy is somewhat weakened by the knowledge that this acute bronchial spasm is by no means pathognomic of the pneumococcus, many poisons producing the same result on injection into an animal. The analogy is, however, suggestive.

13. The dissociation brought about artificially in the laboratory by this investigator may be observed to take place naturally in response to certain kinds of environment. Thus, a strain of pathogenic bacteria may lose its power to produce a certain lesion or to cause a certain symptom in the body. Further, the conditions which appear to deprive it of such functions are comparable to those which, we have seen, influence ferment activity. A few examples will suffice.

(*a*) The quality of *light* to which a culture of bacteria is exposed may modify their power to produce pigment. Exposure to the ultra-violet rays is found to alter profoundly the lesions and symptoms caused by *B. anthracis* (Henri, 1914).

(*b*) The presence or absence of *oxygen* influences pigment production and the fermentation of sugars by bacteria. Foa (1890) isolated strains of pneumococci from the lung and from the spinal fluid of a rabbit which had died after inoculation

with this organism. The strain from the lung possessed the property of causing, when inoculated into another rabbit, an inflammatory oedema of the skin; the strain from the spinal fluid failed to do so. The strain from the lung, however, when grown anaerobically was deprived of its power to cause this inflammatory oedema of the skin.

(*c*) Growth in a certain *vehicle* may alter the fermenting powers of one organism and the pathogenic powers of another. The fermentation properties of a strain of *B. coli* isolated from cowdung become altered after growth in milk. A milk-borne epidemic of scarlet fever is not infrequently characterised by the partial or complete absence of the usual rash.

(*d*) An analogy may also be traced between the *action of chemical substances* added to culture media and the effects of drugs administered in disease. For example, the presence of sodium benzoate inhibits the power of *B. coli* to produce gas from dextrose—one of the most stable and fundamental differences separating *B. coli* from the typhoid-dysentery group—without in any way affecting its other fermenting reactions. The administration of sodium salicylate in rheumatic fever eliminates the symptoms of pain and fever—the two most characteristic symptoms of this disease—without apparently affecting any other of its symptoms and lesions in a great many cases.

14. If the foregoing considerations suggest that the symptoms of disease are due to zymotic action they likewise imply that each separate symptom is attributable to the activity of a distinct enzyme. Such a conclusion postulates the existence of innumerable pathogenic enzymes each one concerned in the causation of some particular symptom of disease, and requires us to conceive of different groups or combinations of enzymes associated with different pathogenic organisms and responsible in the case of each organism for the train of symptoms that follow its invasion of the living tissues.

Analogy with the sugar-fermenting properties of bacteria renders such a complex picture of the causation of disease less fanciful than, at first sight, it appears. As we have shown

(*vide* p. 60) it can be proved that not only is the fermentation of different carbohydrates effected by distinct and appropriate ferments but each of the several stages in the fermentation of a single carbohydrate—such as the formation of acids and the production from these acids of gas—is carried out by its distinct and appropriate ferment. Moreover different carbohydrates yield on fermentation different acids and each different acid requires to be acted on by a special ferment before it becomes split up into gaseous products. If such a comparatively simple result as the production of acid and gas in various carbohydrate media requires the co-operation of so many distinct ferments, the extremely complex and diverse results of the bacterial invasion of the body would appear to demand proportionately greater complexity and diversity in the zymotic agents causing them.

We have seen that the enzymes concerned with the fermentation of particular carbohydrates are definitely associated with certain vegetable and bacterial cells but not with others. For example, many yeasts are able to invert sugar but only three yeasts are known which are able to ferment lactose. Proteolytic ferments are, likewise, associated only with certain vegetable cells, such as the papain. Proteid-splitting and lactose-splitting ferments are associated with certain bacteria of the typhoid-coli group but not with others. It is conceivable that, in precisely the same way, the agencies responsible for certain definite symptoms in disease might be definitely associated with some bacterial cells but not with others.

A further suggestion occurs to one at this point. If the enzyme responsible for one particular symptom of a disease can be dissociated from the specific organism of that disease, should we not expect to be able, by suitable methods, to dissociate from the organism not one only but all the enzymes causing the various symptoms of the disease in question?

15. If such a complete dissociation were practicable it should be possible to accomplish two things; in the first place, to deprive an organism of its power to produce a single one of the symptoms of the disease associated with it and, in

the second place, to reproduce faithfully the complete train of symptoms and lesions characteristic of a disease in the entire absence of the specific organism to which the disease is commonly attributed. Both these results have actually been observed. As regards the first, numerous examples have already been given of virulent organisms, normally capable of giving rise to a complex and characteristic train of symptoms and lesions in the living body (e.g. the Klebs-Loeffler bacillus, *B. typhosus*) being deprived of their power to produce a single one of these symptoms or lesions although, in every other respect, retaining their character and properties unchanged (*vide* "Virulence," "Pathogenesis").

It is well recognised, for instance, that infection with *B. typhosus* may occur without any of the clinical manifestations of typhoid fever. Dudgeon (1908) quotes three cases of patients whose stools contained enormous numbers of typhoid bacilli and whose blood agglutinated these organisms in dilutions of 1 in 200, who nevertheless failed to exhibit a single symptom of typhoid fever.

With regard to the second, examples may be cited of diseases associated with the presence of certain bacteria but now generally recognised as being due to "filter passers." Hog cholera, for instance, is a highly contagious disease associated with a certain bacillus, the "hog cholera bacillus." A pig suffering from the disease can infect other healthy pigs; the latter develop the same symptoms and are found to be invaded by the same organism and they are capable, in their turn, of infecting other healthy animals in precisely the same way. It has been shown, however, that a broth culture of the hog cholera bacillus, from an infected animal, after it has been passed through a Chamberland filter—a process which entirely removes any bacilli present—nevertheless retains its power to "infect" a healthy animal with hog cholera, the disease running the same course as usual and exhibiting precisely the same lesions and symptoms.

Such a sequence affords a precise analogy to the experiment of Sortinin, a quarter of a century ago, which led to his discovery that after a culture of certain bacteria had been

passed through a Chamberland filter, a bacteria-free filtrate was obtained which nevertheless retained the power of the original culture to liquefy gelatin.

16. One objection may be urged at this point, namely, that it has not hitherto been possible to separate from any pathogenic organism an enzyme capable of producing, outside the living body, the toxins characteristic of that organism. It is, however, equally impossible in many cases to isolate from bacteria agents which will bring about other of their functions which we recognise to depend on ferment action. Moreover, we have discussed under the head of virulence (*vide* p. 77) some of the qualities in which artificial media differ from the vital fluids of the body and such differences may well prove an insuperable obstacle to the performance by an enzyme of its usual functions. A pick-pocket may ply his "trade" vigorously in a busy crowded thoroughfare and yet a few hours later, in a workhouse ward, give no sign of his peculiar abilities. In the latter situation certain things are lacking—the incentive which normally stimulates him (that is to say, the "struggle for existence"), the materials he seeks to gain, the conditions essential to his work—and this fact may render difficult if not impossible any display of his customary activities.

The enzyme theory of disease is not at the present stage of our knowledge capable of proof. The above considerations, however, lend some measure, if not of certainty at least of probability to the supposition that the organisms associated with certain diseases are not themselves the causal agents of those diseases but merely act as carriers of ultra-microscopic bodies, possibly parasitic in character, which have hitherto eluded detection but which are the real causal agents of the lesions and symptoms produced.

17. If such an hypothesis should ultimately prove to be correct, how would it affect our ideas as to the possibility of transmutation occurring amongst bacteria? Obviously, if it is possible for the enzyme or enzymes which produce a certain disease to become dissociated from the organism to which that disease is commonly attributed and to become attached to some

other organism, the effect, though not the actual process, of transmutation would be brought about.

A transference of this kind would present certain difficulties. The enzymes—if such be their true nature—of disease would appear to depend, to some extent, for their activity upon the structure and metabolism of the cell body to which they are attached and if they are to be transferred from one organism to another without loss of function the second host must possess those characters in the way of structure and metabolism which are vital to the activity of the enzymes. This implies certain, and possibly rigid limitations. The problem can best be illustrated by analogy with more familiar things.

We are able to distinguish at sea, a fleet of fishing smacks, a line of battleships, a couple of pleasure steamers, a solitary four masted barque in full sail. We distinguish these different types of vessels readily from one another by characters analogous to the "morphology" of bacteria, that is to say their size, shape, motility and grouping. We have, however, another way of distinguishing them, namely by observing the effects produced by their arrival at a port, analogous to the effects of bacterial "invasion." The arrival of the fleet of fishing smacks is followed by a rush of people from their houses to the shore (comparable to the exudation of leucocytes), a silvery deposit on the quay-side as they empty their fish, replaced in a few hours by a brownish membrane as the nets are spread out to dry. The train of "symptoms" is invariable and becomes associated in our minds with the entry into port of this type of vessel. So, too, with the others. The appearance of gunboats may be followed by the destruction of a town (comparable to necrosis). The arrival of the pleasure steamers may be greeted with a display of fireworks (comparable to pyrexia), that of the tall barque with its cargo of spirits may give rise to general intoxication (comparable to delirium).

Such a sequence, however, is not invariable. For example, the fishing smacks might be employed in smuggling and land a cargo of spirits, giving rise to intoxication on shore. The gunboats might be employed by Royalty on a pleasure cruise

and their arrival be greeted with fireworks. A couple of innocent looking steamers might be engaged in piracy and open a destructive fire from their guns. The tall barque might conceivably land a cargo of fish. In other words each type of vessel might give rise to a train of events rightly regarded as characteristic of an altogether different type, for the effects they produce depend not on the activities of the ships themselves, which are merely carriers, but on those of their occupants.

At the same time the function of each different type of vessel, though dependent upon its occupants, is also to some extent governed by its structure and the equipment it carries (comparable to the structure and metabolism of a micro-organism). A mere exchange of crews would not necessarily effect an exchange of function. For example, a party of fishermen sent to sea in an ironclad would be as unlikely to land a catch of fish as a force of naval officers and seamen embarked in fishing smacks would be to bombard a town.

In one respect our analogy fails. Hitherto we have spoken of the enzyme as something grafted on to the micro-organism, in the nature of a parasite, but there is much to suggest in the evidence we have quoted that it is, in reality, a body elaborated by the organism itself, comparable to one of Ehrlich's "side-chains." Such a conception of its nature would go far towards explaining the apparent dependence of the "enzymes" of a particular disease upon a particular organism. But every argument in favour of such a supposition in the case of the enzymes which cause disease applies equally to our conception of the nature of those which ferment carbohydrates. The purpose of the arguments here presented has not been to explain the precise nature of these ultra-microscopic bodies but merely to show that the lesions and symptoms of disease may with some confidence be attributed to the action of the same class of body as that to which we unhesitatingly attribute the fermentation of sugars.

CHAPTER XII

CONCLUSIONS

1. Variation occurs in every character of bacteria.

2. These variations may be either "spontaneous" or "impressed" by conditions of environment.

3. The recognition of "species" amongst bacteria must, therefore, depend upon a consideration of their biological characters as a whole and upon the stability these characters display.

4. Transmutation differs from variation in degree alone; it is a question of the extent of the modification and the degree of permanence it exhibits.

5. Transmutation differs from evolution in degree alone; it is a question of the rapidity of the change.

6. The occurrence of transmutation between closely allied organisms in the human body is not capable of proof but is suggested by circumstantial evidence.

7. Supposed instances of transmutation, brought about by experimental inoculation of animals, are shown to rest on inconclusive evidence.

8. The Enzyme theory of disease suggests a means by which bacteria may exchange many of their characters and functions without themselves undergoing transmutation.

APPENDIX

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