THE REPRODUCTION OF AEROBIC BACTERIA.

By EDWARD C. HORT, F.R.C.P. (Edin.).

(With Plates IV—VII.)

PART I.

Until the year 1916 it was generally believed that the non-sporing bacteria of the lower orders are only capable of reproducing themselves by the simple process of transverse binary fission into two equal parts. This—it was taught—is the beginning and end of their reproductive life. A bacillus, or a coccus, always arises directly from an equally dividing bacillus or coccus, and in no other way whatever. So firmly fixed was this conception of the entire reproductive life of the lower bacteria under all circumstances that any evidence suggestive of the occurrence of other methods of reproduction, such as by budding, or by branching, or by the production of endobodies was apt to be explained away either by alleged contamination of the cultures employed, or by vague references to the phenomena of involution.

In 1916 and 1917, however, and again in 1918, I showed by several series of warm-stage studies of isolated organisms of the enteric group that under certain circumstances the lower bacteria are able to reproduce themselves by the production of fertile branches and buds, and by the endogenous production of gonidial bodies, in addition to the more familiar method by equal binary fission. And I also showed that evidence of this can be found, if looked for, in young cultures in standard media. The cultures I employed were, as I thus proved, pure cultures, so that any suggestion of contamination organisms being present to account for the results obtained was out of the question. And as I had shown that organisms undergoing branching or budding were often fertile organisms, the detached buds and branches themselves giving rise to a new race, it was no longer possible to advance the theory that these were involution forms—in the ordinary sense of the term—since organisms undergoing genuine involutionary changes, recognizable as such, are sterile organisms. The thesis that the lower bacteria can reproduce themselves in more ways than one, when occasion arises, was therefore fully established for the first time, there being no previous record of continuous warm-stage observations to show that isolated organisms belonging to the lower orders of bacteria, and undergoing branching or budding, or endogenous chromatinolysis are capable of perpetuating a new race which is itself fertile, its progeny being culturally, biochemically and serologically identical with the original mother-cell.

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Of the experimental observations establishing these facts several were published in 1916 and in 1917. They therefore need not be reproduced here. Of the remaining observations, and the most complete, a few were examined by a War Office Committee (*v.* note *infra*) specially appointed for the purpose, and are now published—together with further observations—for the first time.

INVOLUTION FORMS: GENUINE AND SPURIOUS.

Before presenting the chief facts on which the above statements are based it is necessary to attempt a definition of the vague term involution form, and to show, in passing, how the literature of bacterial morphology is pervaded with misconceptions arising from the lack of any clear understanding of what an involution form, properly so-called, really means.

An involution form of bacterium can only mean a bacterium which is undergoing retrogressive, or perhaps, degenerative changes. It is, strictly speaking, a sterile organism which is not only incapable of maintaining its reproductive activity, but is also incapable of maintaining its integrity of form. It stains irregularly, feebly or not at all: and in the unstained condition it rapidly fades from view as death ensues, and as autolysis proceeds. It becomes misshapen and deformed, quite early—in the case of motile organisms —losing its motility, as well as that perfect symmetry of outline, and diffused receptivity of stain which mark the healthy normal organism. To the trained observer it offers no difficulty of recognition, and is clearly an organism that has failed in the race of life, as a result of profound disturbance—set up by prolonged sojourn in unfavourable environment—of that power of adjustment of osmotic variations on which all cell activities ultimately depend.

There are, however, often to be seen under less unfavourable conditions aberrant types of the lower bacteria, aberrant that is in the possession of buds or branches, or in some other way, which show none of the characteristics of the genuinely involuting organism. They exhibit perfect symmetry of form, they show no irregularity of staining, which is often deep—especially with carbol-fuchsin—and they do not fade from view in the unstained condition. These organisms are none the less often indiscriminately ranged with genuine involution forms, and are often figured as such, when they do not escape detection altogether, in some of the text-books. So general indeed is the tendency to apply the term involution to any form of bacterium departing from the usual that the legend is unblushingly transcribed from book to book. And the result has been that until recently no effort has been made to apply the crucial test of isolating healthy looking bacteria of the lower orders undergoing branching or budding, and of then determining their ability or inability to produce a new race culturally, biochemically and serologically identical with normal organisms. If an aberrant type of bacterium—neither bacillary nor coccoidal—can be shown to be endowed with fertility it is clearly not an involution form in any reasonable sense of that ill-used word. And yet till

1916 there were no adequate observations on record that this, the supreme test of bacteriological science, had been applied to the lower bacteria to determine whether the current belief in their ability to divide by equal binary fission only was, or was not, well founded: though in the case of other organisms, the existence of higher orders of which appeared thus to be established, the test had already been successfully applied. No effort, in short, was made by applying scientific methods of precision to separate false involution forms from genuine.

For example within the past 25 years several papers have appeared dealing with aberrant morphological types of the lower bacteria, some of the authors, such as Lehmann and Neumann in 1896, on insufficient evidence regarding the occurrence of branching and of budding as a phase of normal development. Others, such as A. Fischer in 1897, and Migula in 1900, regarded the occurrence, again without offering satisfactory evidence, as a pathological process induced by cultivation in unsuitable media. Others again record their observations without comment, or without offering any evidence allowing of interpretation of the results shown. In most of the recorded observations the one constant factor in the media employed for demonstrating morphological aberrancies, indiscriminately classed as involution forms, has apparently been the presence of free H ions, both from organic salts and from inorganic, or the addition of relatively non-toxic doses of the various aniline dyes, perhaps acting in the same direction. Thus Vedder and Duval in 1901 noted that in cultures of dysentery bacilli on glucose agar aberrant types were sometimes found which, without further investigation, they thought were exclusively involutionary in character. In 1900 Fischer recorded that he had placed the *V. cholerae*, the *B. anthracis*, and other organisms in hypotonic solutions of salt in water containing glycerin, and he described a condition to which he gave the term plasmoptysis. Spherical swellings appeared, anywhere in the bacillary axis, filled with plasma extruded from the mother-cell, the plasma pushing the cell-wall, at a weakened spot, he said, in front of it. Some of these spherical swellings gradually expanded and faded from view after varying periods of immersion. In these extreme cases there can be little doubt that the process was the result of a lethal disturbance of osmotic equilibrium set up by prolonged immersion in a fluid with a lower concentration of solutes outside the cell than within it. But Fischer produced no evidence that organisms undergoing lesser degrees of plasmoptysis, in virtue of immersion for shorter periods of time, had lost their reproductive powers. And he produced no evidence to show whether the new spherical bodies produced by plasmoptysis were themselves fertile or sterile. And he therefore provisionally concluded that all degrees of plasmoptysis are necessarily involutionary in character, although he admits that he had not tested the point. To this view also Abbott and Gildersleeve, writing in 1904, in an essay marked by sound critical acumen, were also inclined in their explanation of the occurrence of branching and budding forms in their acid cultures of the *B. diphtheriae*:

though they too were careful to state that they had been unable to determine whether their buds and branches were or were not capable of producing a new race, a criterion to which they evidently attached great importance. In 1904 Ainley Walker and Murray noted the occurrence of branching forms of the *B. typhosus* in media containing gentian violet, fuchsin-methyl green, or methylene blue, and in 1912 Revis dealt with the cultivation of coliform organisms in media containing malachite green. In addition to these there are numerous other recorded observations of morphological aberrations, notably those by Almquist, by Sopp, and by Norsk, to which access has so far not been possible, by Horrocks in 1911, and by Löhnis in 1916.

In all these papers however, with the possible exception of those not yet studied, there is, as stated, no single observation on record that any of these branching or budding forms had been isolated with a view to determining not only their own fertility, but also that of the buds and branches themselves, either before or after their separation from the mother-cell. In addition to all these observations on the occurrence of plasmoptysis phenomena it has not infrequently been noted that under certain conditions plasmolytic changes may be set up in bacteria, leading to endo-fragmentation of chromatin. In bacilli and in cocci so affected, and the change may also be demonstrated in streptococci and streptobacilli, minute dots of deeply-staining material make their appearance. This can often be readily made out in unstained living preparations, which may also contain these bodies in large numbers after extrusion. Photographs of drawings of these bodies were shown by me to the Royal Society in 1916. This endo-fragmentation, described by Fischer in connection with his studies on bacteriolytic serums, has again generally been looked upon as a purely involutionary phenomenon, there being again no recorded warm-stage observations to determine the sterility or fertility of these minute bodies after extrusion from the mother-cell.

PHYSIOLOGICAL AND PATHOLOGICAL DEGREES OF PLASMOPTYSIS AND PLASMOLYSIS.

From what has been said it is clearly a matter of fundamental importance to determine whether the occurrence of plasmoptysis in bacteria is a pathological process only, or whether it is, if disturbance of osmotic control be not too profound, a genuine physiological process. Demonstration of unfamiliar methods of reproduction of organisms undergoing moderate degrees of plasmoptysis, combined with demonstration of the fertility of the new race, would unquestionably prove that the phenomenon does represent a phase in physiological development when environmental conditions become difficult, and, incidentally, that bacteria can reproduce themselves in more ways than one. The problem as to the exact point at which physiological processes end, and pathological processes begin, may safely be left to the curious in such matters. It also appears to be a matter of equal importance to determine whether the occurrence in bacteria of the opposite phenomenon of plasmolysis—induced

by osmotic disturbances set up by a lower concentration of solutes within the cell than outside it—is a pathological process only: or whether it too, when kept within proper limits, does not represent a phase in physiological development.

In the case of plasmoptysis where the crucial point in distinguishing between physiological and pathological events appears to be the potential fertility of the extruded plasma in its new cell-wall, so in the case of plasmolysis the crucial point in giving the correct answer appears to lie in proving or disproving the potential fertility of fragmented chromatin within the cell itself, or outside it. In the former case, that of plasmoptysis, the extruded elements would appear to be the expression of exogenous methods of reproduction by unequal binary fission, giving rise to fertile branches, buds and spherical bodies. And in the latter case, that of plasmolysis, the fragments of intracellular chromatin would appear to be the expression of endogenous reproductive activity equivalent in effect to the exhibition of multiple gonidia: the fertility of the new bodies produced both by exogenous and by endogenous methods of reproduction being finally expressed in the ordinary vegetative forms familiar to laboratory students.

In the appended photographs of drawings will be seen some of the numerous types of organisms observed in 4 per cent. glucose agar, or 4 per cent. glucose broth, cultures from a single colony isolated from the faeces of a severe case of typhoid fever which proved fatal on about the fourteenth day of the disease. Indisputable evidence of the direct relationship of many of these forms to the *B. typhosus* is given below.

PLATE IV.

The drawings in Plate IV represent composite selected fields, that is to say, they do not represent fields as actually observed, each drawn field containing selected organisms from numerous fields, the basis for selection for each field being merely the type of organism it is desired to illustrate. Each field is designated with a separate label, with the suffix -oid, merely to show how closely many of the types depicted may simulate parasitic fungi, and to facilitate description.

For example:

Field 2 illustrates various types of bacteroid
„ 3 „ organisms resembling sporangia
„ 4 „ „ „ chlamydospores
„ 5 „ „ „ oidia
„ 6 „ „ „ gonidia
„ 7 „ „ „ cocci

Fields 1, 2, 3, 4, 5, 9, 10 mainly represent unstained living organisms, drawn, with the exception of 3, by the aid of the camera lucida.

Fields 6, 7, 8 represent stained organisms drawn mostly by freehand.

The following types were found to be highly motile, this motility being repeatedly confirmed during the eight months that this strain was under daily subcultural observation:

Bacteroids
Sporangioids, attached and detached
Chlamydosporoids
Oidioids
Gonidioids.

The only types in which motility was not observed were the thick-walled resting cells depicted in Field 1, and the thin-walled coccoids seen in Field 7. The motility of these there is no record of.

As noted in Plate IV, each field represents organisms observed in cultures on plus 10 agar varying in age between 1 and 4 hours, subcultured from 4 per cent. glucose broth cultures varying in age between 12 and 18 hours. In all cases the maximum incubator temperature employed was 28° C. to 30° C.

The following short description of each field will be sufficient to bring out the points it it desired to emphasize.

Field 8 is of an ordinary plus 10 broth 8 hour *B. typhosus* culture of a strain which had never been grown in media containing glucose. In addition to "normal" bacilli, and one or two organisms exhibiting Artaud's nodes, are to be seen three deeply-staining forms, two bacillary and one bacteroidal, such as are discussed in full in the text. In these deeply-staining forms, which—as here—may or may not be aberrant in form, lies the key to the problem presented by aberrancy of bacterial form. Hitherto these deeply-staining forms, often known as giant-forms, have been looked upon as involution forms, carrying the suspicion of being sterile forms. Actually they are highly fertile, and may exhibit one or more of many different types of reproductive activity. Possession of this deep receptivity of stain appears in fact to presage an explosion of reproductive activity. These organisms are readily made out in the living unstained condition, and can usually be relied on to demonstrate their fertility on the warm-stage when environmental conditions are suitable.

Field 2 represents various types of bacteroid, such as found in *B. radicola* and other organisms of agricultural interest, found in cultures of the single strain of *B. typhosus* here studied. The motility of these bacteroid forms was frequently of the rotatory type, the two limbs of the Y at 28° C. revolving with great rapidity. On detachment of a limb this, whilst still short, moves in the ordinary way as does the ordinary bacillus, but as it lengthens it may take on a well-defined serpentine movement. Some of the bacteroids segment terminally, as shown, in oidial fashion, the small detached spheres being themselves highly motile.

Field 3 represents organisms apparently undergoing the plasmoptysis changes described by Fischer. A normal motile bacillus will sometimes be seen to extrude, anywhere in the bacillary axis, a minute spherical swelling. This gradually expands, the motility of the mother-cell being still unimpaired, progression being in the ordinary way, or by rhythmical serpentine movements. As the sphere expands the mother-cell, with its attached sphere, rapidly rotates, the sphere, if laterally placed, appearing to be presently swung off at a high velocity. Once detached the sphere exhibits a high degree of motility of its own. Its subsequent history is dealt with below. The mother-cell is meanwhile still motile, and may extrude a second, or even a third, new sphere which behaves as before. Sometimes, especially if a sphere has come into contact with another organism, the sphere will remain more or less motionless, whilst the mother-cell will revolve in all planes round the point of attachment to the sphere till finally separation is achieved. If these changes are watched in glucose broth on glucose agar, instead of in normal broth on plus 10 peptone agar, the spheres as formed, presumably on account of the rapidly rising acid tide, will expand and either burst (stained impression films show this well), or will gradually fade from view, either before detachment from the mother-cell, or after detachment, in either case losing their motility.

Field 4 represents types of organisms which are often figured in text-books as "involution" forms, especially those bearing a superficial resemblance to chlamydospores. There are also figured in this field two ovoids. All the forms here shown are highly motile.

Field 5 represents terminal and median segmentations of spherical form, occurring in bacilli, and mimicking oidial formation. The separated coccoidal bodies are motile.

Field 6 shows bacillary, spherical and bacteroid forms undergoing chromatinolysis, the minute fragments of chromatin being frequently observed on the warm-stage in the process of extrusion from the mother-cell. Their subsequent development is dealt with below. Some of the extruded bodies are very minute, often measuring 0·1 to 0·2 microns in their greatest diameter, and able, as such, to pass the coarser filters such as Berkefeld's and Masson's. These minute bodies exhibit high degrees of motility.

Field 7 shows various sizes of coccoidal bodies, mostly with thin walls, when the stain is taken relatively slightly, contrasting strongly with the thick-walled, deeply-staining coccoids, a few of which are seen in Field 1. Photographs of these two types of coccoids are seen in Part II of this paper. It is often said that these coccoid bodies are bacillary bodies seen in cross section. This view is the result of want of their observation on the warm-stage, when they may be readily observed to rotate, if pressure on them be sufficiently light, as definitely spheroidal bodies.

Field 10 represents, grouped in one field, the various types of organism, which were often seen to be present at the same moment in a true microscopic field at the edge of warm-stage preparations of 4 per cent. glucose broth cultures on plus 10 agar. They were also often seen in subcultures in plus 10 broth from glucose cultures. In this field also are seen four forms resembling spirochaetes. These are large detached flagella.

Field 11. These spirochaetoid forms, often tapering to a fine point at each end, with wide spirals, varying in number from 4 to 5 to 70 and 80, the maximum counted (perhaps attached end to end), sometimes stretching across several fields, do not appear to be motile or to segment. They appear in fact to be flagella, attached forms being also shown. They may be seen on the warm-stage to adhere together one by one till thick tresses are formed, such as are sometimes seen in tetanus cultures. They are easily seen unstained, and have been noted in attachment to all the various types of organism shown, including the minute gonidial forms, except to the coccoid forms, and the thick-walled resting cells. The absence of flagella in these forms, appears, as will be seen in Part II, to be associated with non-agglutinability, their development into bacillary forms perhaps going hand in hand with the acquirement of flagella and of agglutinability. It is certain that when development from coccoid to bacillus is complete flagella are present, and that agglutinability is developed, but it has so far proved impossible to watch on the warm-stage the development of flagella *de initio*. In staining preparations of glucose cultures containing large numbers of flagella, attached and detached, it is not necessary to use silver salts. With a preliminary clearing with acetic acid and formalin beautiful preparations are readily made by counter-staining with carbol-fuchsin. In some preparations a fine reticulum may be seen of extremely fine short flagella in dense meshwork formation.

It is necessary to emphasize the fact that all the types of organism shown in Plate IV, except the thick-walled cells, and the large flagella, can frequently be noted in many, but not in all, quite young cultures of the *B. typhosus* in standard media, to which glucose has never been added, provided that patient search be made. It is also necessary to emphasize the fact that the various types of organism shown in Plate IV were again and again found in cultures from single cells, as well as in cultures from single colonies, the precaution always being taken continuously to watch development from single cell to single colony, for purposes of subsequent identification. The necessity for this precaution, and the uselessness in work of this nature of any method of single cell isolation which does not allow of continuous observation from single cell to single colony, will be readily understood by reference to Field 6, Plate IV, and Field 8, Plate IV. The presence of the minute gonidial bodies there depicted makes it impossible to be certain, in the attempt to isolate a single cell of normal bacillary form and size for example, that a minute gonidium is not at the same time being unconsciously picked up. If therefore the ordinary methods of isolation be employed, such as Barber's method, Malone's method, or the fragmented glass method, methods which

involve inoculation of a liquid medium without opportunity for adequate control observation of growth from single cell to single colony, it may happen, and does happen, that more than one organism is picked up, and that the experiment is ruined. If however a method be adopted which does allow of continuous observation to ensure that a given colony has developed from one cell, and from one cell only, the results obtained can be relied on. No satisfactory method of this nature was available, and one had therefore to be devised. This method is fully described in my paper on pp. 361—368 of this volume of *Journ. of Hygiene*.

Plates V and VI.

These Plates reproduce drawings of continuous warm-stage observations of development from single organisms to single colonies.

In *Series* A, B, C, Plate V, and in *Series* I, Plate VI, is figured the development of bacteroids, unequal binary fission giving rise to ordinary bacilli which, as in Plate V A, may continue to divide by equal binary fission, or, as in Plate V D, again become triradiate, and again undergo unequal binary fission. As a rule only one limb of the Y is thrown off at a time, though segmentation of two or three limbs may go on simultaneously, or in short succession. It sometimes happens that a bacillus will undergo unequal fission without manifesting branching.

In *Series* D, Plate V, is seen the development of bacteroid forms from a diplococcal organism, the exact contour of which was determined by observation of rotatory movements *in situ* before elongation to bacillary form had begun.

In *Series* G, Plate VI, is seen development from a single coccoid form to ordinary bacilli.

In *Series* H, Plate VI, is seen development of a "chlamydosporoid" form, germination, segmentation of the new limb, separation, and germination of a fresh limb, with segmentation by equal binary fission of the limb first formed, being clearly seen. This was a highly motile organism, and no attempt was made to immobilize it in order to obtain a camera lucida drawing for fear of arresting its development, a free supply of oxygen having been found essential to rapid growth and segmentation of similar organisms. A freehand drawing was therefore alone possible. A similar course of events is shown in Series J, Plate VI.

In *Series* E, Plate VI, to which the same restriction applies, may be seen the plasmoptysis phenomenon described by Fischer. In this case, however, continuous observation on the warm-stage enabled the observer to determine the fertility and motility of the organism in question, as depicted.

In *Series* F, Plate VI, is shown, at the end of the series, plasmolytic fragmentation of chromatin, followed by extrusion of chromatin, and by development of the highly motile extruded bodies into minute bacilli, each of these after a time exhibiting at each pole one of Artaud's nodes with a clear space between. These rapidly enlarged, though the enlargement is not drawn, under observation, still retaining their motility. These are the minute gonidial bodies, figured in Field 6, Plate IV, as present in, and extruded from, bacillary forms as well as from spheroidal, which can pass coarse bacterial filters, and which can sometimes be seen in standard media, as well as in glucose media, or in media to which HCl has been added, when the requisite alteration of concentration of solutes—in the direction of plasmolysis production—has been reached. And whenever very minute motile bacilli, especially when showing Artaud's nodes, appear in pure cultures of normal sized *B. typhosus* cultures it may safely be concluded that they have arisen in this way from minute extruded gonidial bodies. In order satisfactorily to study the development of these organisms a free supply of oxygen and of moisture is absolutely essential, no development of this nature taking place except at the moist edge of the preparation nearest to a free supply of air.

Having thus shown development from single cell to single colony it was then necessary to identify fully the colonies obtained. Eighteen different aberrant types were isolated, including giant bacillary forms, similar to those seen in Plate IV, Field 8, coccoid forms similar to those seen in Field 7, bacteroids similar to some of those seen in Field 2, chlamydosporoids similar to some of those seen in Field 4, sporangioids similar to those seen in Field 3, and resting cells similar to those seen in Field 1.

The broth cultures from each of these were fully identified by cultural, biochemical and serological tests, an agglutination of from 1/20,000 to 1/40,000 being in all cases eventually obtained.

In two cases identification was further completed by absorption tests, and in the case of the cultures handed to the War Office Committee of enquiry absorption and inoculation tests were also successfully passed.

During the course of the work undertaken in connection with study of methods of bacterial reproduction the following facts came chiefly into prominence. Some of these have already been noted by bacterial morphologists.

1. In some cultures of the lower bacteria, whether young or relatively old, whether standard in initial reaction, or containing glucose from which, in the case of the *B. typhosus*, acid is rapidly formed, or deliberately made acid by the addition of free HCl, aberrant forms of organisms have never been found, even after prolonged search. The entire population in such cases appears to consist of "normal" organisms, staining relatively lightly, and dividing by equal binary fission only. The general viability of such a population after a few days in 4 per cent. glucose cultures, or in HCl cultures with an initial reaction to phenolphthalein of plus 20, appears to be considerably lowered, as measured by subculture.

2. Aberrant types of organism are sometimes to be seen in small numbers in quite young cultures in "standard" media. These aberrant types, found in young cultures, sometimes in relatively large numbers, usually stain deeply and uniformly, unless undergoing chromatinolysis, when the fragmented chromatin appears as deeply staining dots, contrasting strongly with the less deeply-staining mother-cell. These aberrant types may be only aberrant in their deep receptivity of stain, there being no departure from the normal bacillus in outline. Other aberrant types, also characterized by uniformly deep receptivity of stain, may be branching or budding forms, or may be coccoid in form, or may simulate parasitic fungi by the formation of bodies resembling sporangia, chlamydospores and so forth. All these deeply-staining forms may be highly fertile.

3. These aberrant types, if already present in a standard culture, will increase in number with the age of the culture, or by the addition of glucose, or of free acid, the normal population under such circumstances beginning to disappear until it is again temporarily reinforced by direct descendants from these fertile aberrant forms. The viability of such a culture as a whole is greatly impaired by prolonged immersion in 4 per cent. glucose media, or in media containing an excess of free HCl. The viability of the deeply-staining

aberrant forms referred to is however higher than that of the general population, and it is clear that they have higher resisting powers to acid influence. This is also shown by the fact of their presence in greater number in old cultures than in young, and in the fact that on transference to fresh media the new generation appears to start from them and not from the survivors of the normal population. This can readily be demonstrated by watching the development of individual organisms on the warm-stage. It is quite unnecessary in order to establish the higher resisting powers to acid influence of these selected individuals to show also that they exhibit greater resistance to heat or to antiseptics than do "normal" individuals. This statement is necessitated by attempts that have unsuccessfully been made in the past to establish a higher degree of resistance to heat and to antiseptics in the case of so-called "involution" forms, as compared with normal forms, without any reference to the cardinal fact of their demonstrable fertility.

4. In addition to the fertile deeply-staining forms described there are also often to be seen in young cultures in standard media, as well as in glucose media, lightly-staining aberrant forms. These organisms, distinguishable from involution forms in their symmetry of outline, and in the fact that they do not fade from view on the warm-stage, may be themselves highly fertile. Examples of these in coccoid form are seen in Chart 4, Part II, of this paper.

5. On transference to standard media from glucose cultures, or from HCl cultures, aberrant forms tend rapidly to disappear, the need for their services being perhaps no longer required in early stages of growth on standard media. When, however, the age of these media increases, or when glucose is added, they again tend to reappear. And if careful search be made it will often be found, whilst the cultures are still young, or before glucose has been added, that deeply-staining bacillary forms are here and there present. So long as conditions remain more or less favourable these may divide by equal binary fission only. But when conditions become less favourable they may often be seen to take on unfamiliar methods of reproduction, though this, as shown in Plates V and VI, is also to be witnessed within an hour or two of transference to standard media.

6. Transference of genuine involution forms from old or otherwise unsuitable media to fresh standard media does not restore their uniform receptivity of stain, their symmetry of outline, nor their fertility. In some instances there may be seen to appear in genuine involution forms highly-refractile spherical bodies, suggesting droplets of lipoid material, which rapidly increase in size under observation. These bodies do not take the ordinary stains, but stand out as bright clear spaces in the irregularly stained matrix of the cell.

7. If standard broth media with an initial reaction of plus 10 to phenolphthalein be inoculated with acid-producing organisms, such as the *B. typhosus*, and be repeatedly titrated, evidence of a rapidly rising acid tide is forthcoming within two or three hours of incubation. A progressively unfavourable environment is thus rapidly produced which appears to be similar to, and to approxi-

mate to, the unfavourable environment of old cultures, or of cultures to which glucose or free acid has been added. The rapidly increasing concentration in a constant volume, with no provision for their removal, of degradation products from the substrate presented by the constituents of media, and by dead organisms, with a rapidly increasing concentration of organic catalysts no doubt accounts for this rising acid tide in standard media. In attempting therefore to estimate the reciprocal influence of bacteria and their surroundings in terms of morphological results it is obvious that the term "standard medium"—once inoculation has taken place, and once the population has begun greatly to increase—has no actuality.

CONCLUSIONS.

In the light of the facts here recorded it would appear that from the point of view of perpetuation of the lower bacteria through long periods of time, in laboratory cultures at least, the organisms usually regarded as "normal," normal, that is, in form, in their relatively slight capacity to retain the stain, and in their exclusive ability—exclusive so long only as conditions remain favourable—to divide by equal binary fission, represent the least important members of a total population. These "normal" organisms occur in the largest numbers in a given culture when the reaction to phenolphthalein approximates to the neutral point: and it appears that in some cultures they represent the entire population. When, however, as the result of rapid increase in numbers, the circumstances of life become more and more adverse the onus of carrying on the race appears to be chiefly laid on those deeply-staining highly fertile organisms which, from too ready an assumption of their sterility, have hitherto been indiscriminately ranged with genuine involution forms. From the point of view of perpetuation of the race as a whole these deeply-staining organisms, sometimes aberrant in form and sometimes not, able to produce fresh bacilli now by this method of reproduction, now by that, according to the osmotic needs of the moment, are incomparably the most important. In the history of bacteria of the lower orders it would appear that the existence of these deeply-staining organisms, and of the various types of reproductive activity which they exhibit, is a direct expression of the reciprocal influence through the ages of bacteria and their surroundings in terms of selective adaptation to the vicissitudes of bacterial life (unless indeed it be alternatively suggested that they represent a non-bacterial order, with equally dividing "bacteria" as a vegetative side-issue, as from their mimicry of the parasitic fungi one was tempted to surmise). And if it were not for these deeply-staining forms, with relatively high viability in acid media, it would be difficult to see how cultures of non-sporing bacteria could survive in the test-tube for long periods of time if reliance could only be placed on "normal" individuals with the lower degree of viability which is unquestionably imposed on them by prolonged residence in progressively adverse surroundings. It must not however be supposed that the process of selective adaptability is one which

can be followed at will in the laboratory and that "forms identical with the aberrant forms here depicted have arisen in definite response to the conditions experimentally provided, and may be expected constantly to reappear whenever the same environmental conditions are reproduced," as suggested by the Committee selected to study the facts presented. Because this is precisely what does not happen. As already stated these deeply-staining aberrant fertile organisms only appear to increase in cultures already containing them, as for example in cultures in which circumstance has deliberately been made adverse. They emphatically do not appear, within the limits of observation, in populations exclusively composed of normal organisms when the same adverse circumstances are introduced. One can only conclude therefore that in these selected individuals there resides, as the result of far distant training, the inherited faculty of reproduction by unfamiliar methods when necessity arises. And these methods are only unfamiliar because this has not been recognized, and because they have not been looked for.

NOTE.

This Committee consisted of Colonel Sir William B. Leishman, Colonel Professor J. G. Adami, Professor J. B. Farmer and Lieut.-Colonel D. Harvey.

The subject of enquiry was presented by me in a typed memorandum in the following terms:

> "The sole objective of the present enquiry is determination of the complexity or otherwise of the life-cycle of the causal organism of Typhoid Fever, in so far as this is amenable to study in unfiltered laboratory cultures. We are therefore here concerned with one question only—Is the *B. typhosus*, or is it not, capable of being produced in any other way whatever beyond that of equal transverse binary fission of a pre-existing *B. typhosus*?"

During their work this Committee applied to my cultures the ordinary routine tests for purity, including absorption and inoculation tests. And they reported that they fully endorsed my statement that the cultures were pure cultures. They did not however apply the crucial test of purity which I had myself applied, namely that of isolation of single aberrant cells, and of continuous observation on the warm-stage of growth from single cell to single colony, with subsequent indentification of the new races thus produced. They also fully confirmed by personal observation of warm-stage development my claim that the *B. typhosus* can be reproduced from non-bacillary forms by unequal binary fission, as well as from bacillary forms by the ordinary method of equal binary fission. Confirmation by independent observers of my main thesis, as specifically presented, was thus complete. It may therefore be unequivocally stated that it has now been definitely proved, and accepted, that the lower bacteria, as illustrated by the *B. typhosus*, can and do reproduce themselves in more ways than one, and that conse-

quently the reproductive life of this organism is not necessarily so simple as it has hitherto been assumed to be.

The Committee, I think wisely, deprecate the use of the term life-cycle in connection with the reproductive life of the individual bacterium as an organism which inevitably, sooner or later, as a necessary stage in the completion of its life-story must—in the case, for example, of a bacillus—pass through a non-bacillary phase. In the case of bacteria placed under the artificially favourable environment supplied for short periods of time by culture media with an initial standard reaction the life-story, such as it is, is unquestionably simple and not complex. But it is not sufficient to study the morphology of bacteria in the short periods when circumstance is easy. It must never be forgotten that this initial state of favourable circumstance is always fugitive in nature, in infected tissues, and in the test-tube, once the population has begun rapidly to increase in a confined space, with no provision for removal of degradation products. It is this progressively unfavourable environment which appears to have evoked a selective adaptation in virtue of which unfamiliar methods of reproduction come into play to ensure permanence of the race through long periods of time. To say therefore that in the lower bacteria the reproductive life is sometimes not simple but complex is indisputably true, because to preserve the race in terms of time a bacillus, for example, may have eventually to pass through a non-bacillary phase.

The confirmation, above referred to, by this Committee of the complexity of bacterial reproductive life under certain circumstances was however at once qualified by an evident reluctance to abandon the idea that the results demonstrated were the expression of an involutionary process, and had in consequence little or no bearing on the practice of bacteriological science.

In support of their view that involutionary processes could still be invoked to explain the results the Committee referred to the work of Fischer, and of Abbott and Gildersleeve, under the evident impression that the plasmoptysis of Fischer was looked upon by these authors as necessarily a pathological process. Fischer however, as well as Abbott and Gildersleeve, specifically state that they had had no opportunity of determining the fertility or sterility of the new bodies produced by plasmoptysis, a distinction which these authors clearly recognized to be one of fundamental importance. And their lack of opportunity of settling this point appears to have been due to the fact that they knew of no reliable method of isolating organisms for the purpose, a deficiency which I had been at great pains to make good. In the absence therefore of demonstration of the fertility of these organisms these anthors had no alternative but provisionally to assume that they were sterile, and to conjecture that all degrees of plasmoptysis are therefore involutionary in nature. The bacteriologists of the Committee however in their official report make no reference to this qualification of Fischer's work by Fischer himself, and by Abbott and Gildersleeve, although they had my own positive evidence of fertility before them, which indeed they confirmed by their own observations.

In their reference therefore to this earlier work as confirmatory of their own view they must, doubtless under the stress of war work, either have failed to note that the crucial point of fertility had not been tested in this earlier work—this being the vital distinction between my work and that of previous observers, a distinction which they also omitted to record—or they had not recognized that demonstration of the fertility of new races abnormally produced is fatal to the involution theory, as generally understood. In either case, as they are careful to avoid the use of the word involution, it is necessary to point out that organisms undergoing pathological degrees of plasmolysis and plasmoptysis are only involution forms under another name, and that there appear to be physiological degrees of these phenomena resulting from disturbances of normal restraint of osmotic changes as well as pathological degrees of the same. It is indeed difficult to see how the occurrence of bacterial branching and budding, and of endogenous gonidia production, can be anything else but an expression of restrained plasmoptysis and plasmolysis, assuming, that is, that disturbance of the power of adjustment of osmotic variations within the cell and outside it is the true explanation of the various types of morphological aberrancy here illustrated—an assumption on which few, perhaps, would care at present to dogmatize.

PART II.

THE EFFECT OF THE COMPLEXITY OF THE REPRODUCTIVE LIFE OF BACTERIA ON THE AGGLUTINABILITY OF BACTERIAL EMULSIONS.

Belief in the ability of the non-sporing bacteria to divide under all circumstances by equal binary fission only is reflected in bacteriological practice, especially as regards determination of the precise etiology of infections, the methods employed for identification of bacteria, and the theory of the multiplicity of bacterial strains. It also enters largely into prophylaxis and treatment with specific serums and vaccines, and is an essential feature in the detection and control of bacterial carriers, as well as in epidemiological work, including statistical analyses. Now however that it is known that the life-story of bacteria, even in standard laboratory media, is one of great complexity, and that their reproductive life faithfully reflects the progressive changes of milieu inseparable from laboratory cultivation it becomes necessary to examine the various bacteriological problems referred to from a different point of view.

In the present communication I propose only to deal with the problem presented by serological reactions as an aid to identification of bacteria, and in doing so to show that it is hopeless to expect to obtain constant agglutination results unless due regard be paid to the morphological status of a bacterial population, both when used as an antigen for the production of agglutinin, and when tested for its power of provoking a specific reaction.

I propose, in other words, to show that necessary as attempts at standardization are in terms of initial reaction, or the opacity of an emulsion, or in terms of constancy of culture medium composition, one cardinal feature at least in standardizing serological reactions is determination of a standard morphological equivalent. Unless this be fully taken into account agglutination units have but little practical significance.

It has of course long been known that bacillary organisms of the lower orders of bacteria may occasionally take on a coccoidal phase, and that this may be associated with a deficiency in agglutinability by the specific serum. And there are well known to occur various vagaries of agglutinability, hitherto unexplained, such for example, as the loss of agglutinability on subculture to an agar slope, with subsequent recovery on again restoring the strain to broth. But, as a rule, the current conception of the simple life-history of bacteria has dominated the identification work of bacteriologists, and has prevented any systematic examination of the morphology of bacterial emulsions which might be expected to agglutinate with a given serum, but which nevertheless do not do so. And in consequence there has arisen, especially in bacillary dysentery, a heterogeneous collection of new "strains," marked by strange symbols, justification for the existence of at least some of which rests on the slenderest evidence. It is not possible as yet, owing to the bewildering complexity of the study of bacterial morphology, absolutely to prove that some of these many strains only represent different morphological phases of one strain, difference in form being associated with difference in antigenic values. Nor is it desired to insist that standardization in terms of morphology is the only cardinal factor to be considered in the standardization of bacteriological methods of serological aids to identification. But, as will be seen, there is abundant evidence to show that the morphological factor is one which demands the most searching study, which no bacteriologist can afford to set aside as unnecessary if the standardization of bacteriological methods generally is to be put on a scientific footing.

The value of the agglutination results here recorded entirely rests on the reliability of the technique employed. It is therefore necessary to give a series of control observations to show that constant results were uniformly obtained in using this technique when an emulsion of the same morphological equivalent was put up against its specific serum.

CONTROL CHART.

The technique employed in all cases was as follows. The method of reading was macroscopic, often confirmed by microscopic, or by hand-lens, examinations. Distribution of equal volumes of saline, serum and emulsion was made with a new graduated Pasteur pipette for each set of tubes used, the pipette being thoroughly washed and drained between each distribution of material. Dilutions in all cases began at 1/80, except when otherwise stated, the dilution in each case except the first being double that in the preceding tube. Twelve

hour cultures were uniformly employed, the test emulsions being put up without killing the organisms. In the case of *B. typhosus* cultures the plugged tubes were placed in a hot chamber for one hour at 56° C., after which time they were allowed to stand for one hour at room temperature when a first reading was taken, a second reading being taken 12 or 24 hours later. In the case of dysentery cultures the tubes were kept for 24 hours at 56° C. No attempt was made to standardize in terms of opacity, a deliberate omission in order to determine the effect of the omission on the control observations. As will be seen in Chart A the effect was nil. A control tube containing saline and emulsion only was put up for each set of observations in all the experiments.

The first set of observations deals with typhoid cultures. This set is divided into the following groups.

Chart 1, Group A.

In the case of strain Pierce, morphological variations in which formed the subject of Part I of this paper, it was found that the thick-walled coccoids there referred to, *vide* Plate VII, figure 1, were non-agglutinable. It was also found that under the influence of the acid produced from the glucose added to the cultures these thick-walled coccoids represented a resistant resting phase, and occurred in a bacillary population which was itself fully agglutinable. It was also found by isolation of one of these thick-walled cells that it germinated, and gave rise to large bacillary forms, which in their turn gave rise to forms which were again fully agglutinable. The process from agglutinable bacillus to non-agglutinable thick-walled cell and back again to agglutinable cell was therefore reversible. The results obtained are expressed in graphic form in Chart 1, which also gives the key to cultures submitted to agglutination tests, together with the dominant morphological equivalent for each culture. Photographs of these thick-walled coccoids are appended. It is noticeable that emulsions 4 and 8 were from glucose agar slopes.

Chart 1, Group B.

From the same strain Pierce subcultures from glucose broth were made as shown in the chart. In cultures 1 and 2 the dominant population was composed of thin-walled lightly-staining coccoids. Photographs of the actual emulsions of these coccoids used in these agglutination tests are given, together with a photograph of the mixed coccoid and bacillary emulsion 3, which agglutinated to 1/1280. The remaining emulsions 4, 5, 6 were mainly bacillary, as also was the original emulsion A, all of these agglutinating to high titre. It is again to be observed that the non-agglutinable emulsions 1 and 2 were from an agar slope, and from a glucose agar slope. Since recovery of agglutinability ensued on subculture in broth, *vide* cultures 4 and 5, associated with reversal to bacillary form, it might be thought that the appearance of a coccoidal non-agglutinable phase is the result of cultivation on a solid medium. That this is not necessarily the case is shown in Group C, where the non-agglutinable phase is seen to persist in broth culture.

Chart 1, Group C.

From the key to subcultures 1, 2, 3, 4, 5 from the same Pierce strain it will be seen, Chart 1, Group C, that the non-agglutinable coccoidal phase persists in broth cultures 2 and 5 with temporary reversion to agglutinable bacillary phase in broth culture 3, followed by return to coccoidal non-agglutinable phase on agar, culture 4: the same persistence of this in broth, culture 5, from the original agar, being again seen.

CHART 1, GROUP D.

Having thus found in Groups A, B, C that, using the stock serum issued by the Lister Institute, a dominant coccoid population in a selected *B. typhosus* strain subcultured from glucose cultures, will show little or no signs of agglutination, whilst bacillary subcultures from single isolated coccoids (*vide* Plate V, Part I) may agglutinate in a dilution of 1/40,960, the same strain Pierce was then tested in parallel with an R.A.M.C. stock serum, and with the same Lister stock serum as before; two colonies being subcultured from the same agar plate, for purposes of comparison, into glucose broth, each tube of this being further subcultured as shown in the key. It was not found possible, at the date on which this experiment was carried out, to provide at the required moment a subculture from strain Pierce with as dominant a population of thin-walled coccoids as shown in Groups C and D. But a sufficient number were demonstrable to produce wide variations in the agglutination results obtained between the readings respectively of dominant coccoidal and dominant bacillary populations, with intermediate readings, discussion of which is for the moment deferred. In addition to this the following points of interest emerge from study of Group D.

A. Subculture 7, in which the coccoidal population was dominant, agglutinated only to 1/320 with both the Lister and the R.A.M.C. serums, in the case of colony A, and only to 1/160 with both serums, in the case of colony B. The amount in both the Lister and the R.A.M.C. serums of specific agglutinin to the coccoid phase of the *B. typhosus* would appear therefore to be very small. In the case of the Lister serum this had already strongly been suggested in groups A, B and C. The great similarity in the agglutination results in the case of subcultures 7, as regards both colonies A and B, also indicate that the course of development in the case of both these colonies, as shown in the particular subculture 7, was approximately the same. And this was confirmed by the dominant coccoid population in both. So far the distinction between coccoid non-agglutinable and bacillary agglutinable phases, already illustrated in groups A, B and C, appears to be a simple matter, when considered in the light of results obtained by using subcultures from a single colony tested against one serum.

B. When however further controls are established, as by testing subcultures from two colonies against two serums in parallel, most discordant results emerge, which suggest that it is not merely a question of the relative percentages of coccoidal and bacillary members of a population. For example, subculture 10, colony A, gives a full titre of 1/20,480 with both Lister and R.A.M.C. serums, whilst subculture 10, colony B, gives a full titre of 1/20,480 with Lister serum, but a titre only of 1/1280 with R.A.M.C. serum. Again, subcultures 1, 2, 4, 6, 8, 9, colony A, give full titre with Lister serum, but much smaller titres with R.A.M.C. serum: whilst, allowing for the smaller number of subcultures tested in the case of colony B, there is again great similarity in the results obtained with Lister serum, but great disparity in the results obtained with R.A.M.C. serum. Imperfect observation of the agglutination results affords one way of escape from the dilemma. This is however excluded by the control results given at the opening of Part II of this paper. Contamination of the cultures affords a second way of escape. This however is practically excluded by the fact that cultures 1, 2, 4, 6, 8, 9, 10 agglutinated to 1/20,480 with Lister serum in the case of colony A, and that cultures 1, 2, 8, 9, 10 agglutinated to the same titre with the same serum in the case of colony B. Still a third way of escape is offered by the theory, upheld by many, that any given bacterial culture may be composed of numerous strains, and that some subcultures from such cultures may exalt this strain, and other subcultures that strain. This way of escape also appears to be closed by the fact, demonstrated in Part I, that organisms appearing to represent different strains in one culture can develop within a few hours from a single cell. The true explanation in fact appears to lie in this demonstration, though it is not suggested that other factors, as yet

undetermined, may not be partly responsible for the agglutination vagaries here illustrated. Confirmation of the view that each agglutination departure from full titre corresponds with a definite morphological equivalent was obtained by careful examination of subcultures 1, 2, 4, 6, 8, 9, 10 when it was found that:

(1) Repeated replating gave no indication of contamination.

(2) Culturally, biochemically and serologically (in terms of Lister serum) they were genuine *B. typhosus* cultures.

(3) Morphologically some of the cultures contained in addition to "normal" bacilli large numbers of forms of bewildering variety, the relative percentage numbers of these—which were mostly bacillary—varying with each subculture. In addition, for example, to coccoids and to "normal" organisms would be seen bacilli considerably larger, and very much smaller, than the normal, some exclusively bacillary, some cocco-bacillary, others undergoing budding or branching, others again undergoing chromatinolysis. Some had pointed ends, others had rounded ends: some were small, exhibiting Artaud's nodes, others were considerably larger, also exhibiting Artaud's nodes. Some were thick-walled bacilli, taking the stain deeply, others, again, of the various types described took the stain relatively lightly.

The amount of work involved in attempting to obtain an accurate idea of the relative proportion of each of these various types in a large number of cultures exhibiting intermediate degrees of agglutinability would clearly be prohibitive, without a trained staff of workers. No attempt therefore was made in the observations now to be recorded to interpret in morphological terms varying degrees of agglutinability of different subcultures of the same culture when tested either with one serum, or with two or more serums in parallel: the main object being merely to show that standardization in terms of morphology must in the future be comprehensively studied if constantly reliable results are to be obtained.

Chart 1, Group E.

That some serums appear to have been unconsciously prepared with polyvalent antigens, polyvalent that is in the sense of polyvalency of morphological types and not in the sense of polyvalency of "strains," is shown by Group E. In this group subcultures from the same strain Pierce were tested against the Oxford stock serum, and against the R.A.M.C. serum. The results, as before, with the R.A.M.C. serum are poor, whilst the results with the Oxford serum, against the Pierce strain, are very good, suggesting lack of morphological polyvalency in the former serum, and its presence in the case of the latter serum.

So far tests have been confined to subcultures of the strain of *B. typhosus* from glucose cultures, which appear to be particularly favourable to the initiation of different types of morphological development. To this extent therefore the tests so far applied would appear to be unnecessarily severe, and perhaps of little practical value unless confirmed by similar results obtained from tests applied to subcultures from ordinary cultures to which glucose has not been added. Tests were therefore applied to subcultures from ordinary cultures, in order to determine if similar vagaries of agglutination occurred, with the following results.

Chart 2, Group F.

Here considerably better results are obtained, though even with such good serums as the Oxford and Lister serums marked variations in the titres given with each of these occur. Taking the results given with the three serums the minimum occurs with the R.A.M.C. serum of 1/640, and the maximum with the Lister serum of 1/40,960.

DYSENTERY CULTURES.

CHART 3, GROUP G.

As shown in this chart four subcultures from 12 hour agar cultures of Lister stock strains F and Y were tested respectively against Lister F and Y stock serums, cross tests being also carried out. In this experiment the condensation water in each agar tube was replaced by broth, cultures 2 and 4 representing the broth substitute for condensation water in cultures 1 and 3. The object of this arrangement was merely to show that different morphological results, with different morphological readings, can be shown in one test-tube according to whether the agar slope, or the broth at the bottom of the tube, be inoculated at the same moment from the same source: care of course being taken to keep the tubes vertical during incubation, and to ensure that in removing the broth culture no admixture of this with the culture on the surface of the agar slope not covered by broth takes place. This agar culture, after removal of the broth, was itself removed with a sterile glass rod from the upper half only of the slope, and was then emulsified in sterile broth: both cultures being then at once tested. Study of the chart shows that:

1. The *B. Flexner* broth subculture 4 does not agglutinate at all, in any of the given dilutions, either with Flexner serum, or with Y serum: whilst the B. Y broth culture 4 agglutinates to 1/160 with Flexner serum, and to 1/320 with Y serum. The approximate respective morphological picture in each of these cultures is given in the chart.

2. The *B. Flexner* agar subculture 3 agglutinates to 1/640, with Flexner serum, and to 1/160 with Y serum: whilst the B. Y agar subculture 3 agglutinates to 1/320 with Flexner serum, and to 1/320 with Y serum.

3. *B. Flexner* agar subculture 1 agglutinates to 1/160 with both Flexner serum and Y serum, whilst B. Y agar subculture 1 agglutinates to 1/640 with Flexner serum, and not at all with Y serum.

4. *B. Flexner* broth subculture 2 does not agglutinate at all, in any of the dilutions, with Flexner serum, but to 1/1280 with Y serum: whilst B. Y broth subculture 2 does not agglutinate at all, in any of the given dilutions, with Flexner serum, and to 1/640 with Y serum.

Thus, to take an extreme example, agglutination of B. Y agar subculture 1 would suggest that this was a culture of *B. Flexner*, other tests being in the same direction, the figures for agglutination being 1/640 with Flexner serum, and nil with Y serum: whilst agglutination of B. Y broth subculture 2 would suggest that this was a culture of B. Y, the figures being nil with Flexner serum, and 1/1640 with Y serum. Taking the agglutination results of all four subcultures as a whole an empirical agglutination mean, E.A.M., can be obtained for each set of four observations with one serum by dividing the total length of agglutination lines by the number of subcultures examined.

The results may then be expressed:

B. Flexner versus	Flexner serum	= 10	E.A.M.
„	„	Y	„	= 13	„
B. Y	„	Flexner	„	= 16	„
„	„	Y	„	= 13	„

The agglutination results, in other words, suggest that, taking the subcultures as a whole, *B. Flexner* agglutinates better with Y serum than with its own serum, and that B. Y agglutinates better with Flexner serum than with Y serum.

Unfortunately reliable sets of sugars were not available when these experiments were carried out: so no opportunity occurred of determining if the results obtained were associated with any biochemical variations. Observations since undertaken, indicating in some cases association of biochemical variation with serological, will be published separately.

Chart 3, Group H.

Observations were then carried out with R.A.M.C. subcultures from stock Flexner and Y cultures, kindly supplied by Lt.-Colonel Harvey, tested against R.A.M.C. stock Flexner and Y serums.

The massed results obtained with six subcultures, instead of four, show a better specificity of reaction in the case of R.A.M.C. cultures and serums than in the case of Lister cultures and serums, the figures being:

B. Flexner versus	Flexner	serum	= 15	E.A.M.
„	„	Y	„ = 10	„
B. Y	„	Flexner	„ = 6	„
„	„	Y	„ = 17	„

A considerable variation is, however, to be noted in each group between the minimum and the maximum dilutions at which agglutination occurred.

Chart 3, Group I.

The R.A.M.C. stock *B. Flexner* culture used for the observations recorded in Group G, was kept in a sealed tube for over three months at 5° C., and was again subcultured as shown in Group H, several replating observations being made to ensure the absence of contamination. The object of the experiment was to determine the effect of subcultivation from an old culture in the direction of inducing in these subcultures a greater, or a lesser, variation in their agglutination figures than in the case of subcultures from young cultures, as in Group G. At the same time it was desired to test these subcultures against a Lister Flexner serum, and against a Lister F Y serum.

For purposes of comparison the figures from A in Group G are reproduced in the present Group H. The E.A.M. for the R.A.M.C. Flexner subcultures tested with R.A.M.C. Flexner serum is now seen to rise from 15 to 42, the maximum titre supplied by the makers being greatly exceeded in the case of cultures 1, 3, 2, 6. The E.A.M. for the same subcultures tested with Lister Flexner serum C 94 is only 5, three of the subcultures giving no reaction at all. The E.A.M. for these subcultures tested with Lister Flexner Y serum, is the much better figure 32, which is of particular interest in view of the fact that this serum appears to have been prepared by inoculation of F and Y antigens, Lister F and Y cultures having been shown in Group A to be more or less interchangeable.

The maximum variations, as for example B—between 1/160 and 1/20,480, again show how nearly subculture 4, for example, came to the minimum diagnostic titre, even when tested against so good a Flexner serum as R.A.M.C. serum F 7.

Chart 3, Groups K_1, K_2, K_3.

This chart illustrates the discordant results obtained when the R.A.M.C. stock Flexner culture was replated, half of a colony being subcultured on to agar, the other half being subcultured into broth, at the same moment. The same Flexner culture was replated eight times, each time on a series of five plates. Each series was lettered in succession, A, B, C, D, E, F, G, H, a single colony from plates 4 or 5 from each series C, D, E, F, G, H being divided as described. There was an interval of three days between each replating, the original culture remaining at room temperature in the interval.

Chart 4, Group L_1.

This chart illustrates the results obtained by testing subcultures, for the most part at random—and not entirely, as hitherto, in groups, from a Y culture, obtained from the Kitchener Hospital, Brighton, against different Y and F Y serums, and against a polyvalent serum. As will be seen the agglutination figures obtained do not, taken as a whole,

in this case indicate a marked advantage in favour of the R.A.M.C. polyvalent serum employed, wide variations, from 1/160 to 1/10,240, of readings occurring, in the case of five subcultures from the original agar when tested with this serum.

CHART 4, GROUP L_2.

In this experiment a normal polyvalent serum, R.A.M.C. polyvalent Flexner Y serum D_1 was tested against:

a. The same Y strain as used in Chart 4, L_1.
b. A second Y strain, also from the Kitchener Hospital.
c. The stock R.A.M.C. Flexner strain, Ledingham, employed in the earlier experiments.

It was desired to determine what variation, if any, occurred when a different medium was employed, such as the tryp. agar medium recommended for meningococcus work.

As will be seen the results obtained with this polyvalent F Y serum were very good, and more constant. The results obtained with agar and with tryp. agar subcultures are also very similar, indicating that the change of medium has little or no effect in producing different degrees of agglutinability.

CHART 5, GROUP M.

Attention was now turned to a stock Shiga-Kruse culture, kindly supplied for the purpose by Professor Dreyer's staff at Oxford, subcultures from this being tested against a stock R.A.M.C. Shiga serum, and a stock Lister Shiga serum.

The results obtained may be summarized as follows:

1. *With Oxford Shiga serum.*
   (*a*) Subcultures 13, 14, 15 give a uniform figure of 1/40,960, suggesting that this is an ideal serum.
   (*b*) Subcultures 19, 20, 21 from the original agar slope—which had been standing for four days at room temperature—now uniformly declined to agglutinate in a higher dilution than 1/80.
   (*c*) Subculture 25 from subculture 19 still persisted in refusing to agglutinate, whilst subculture 28 from subculture 20 agglutinated in a dilution of 1/2,560, subculture 27 from subculture 21 now agglutinating in a dilution of 1/40,960.
   (*d*) Subcultures 31 and 32 from the original agar slope—which had been standing for six days at room temperature—now both agglutinated in dilutions of 1/20,480.
2. *With Lister serum.*
   (*a*) Subcultures 13, 14, 15 which all gave a figure of 1/40,960 with Oxford serum gave with Lister serum figures of 1/80, 1/160 and 1/320, suggesting that the phases agglutinable by Oxford serum are not represented by specific agglutinins in Lister serum.
   (*b*) Subcultures 25, 27, 28 now agglutinate to 1/10,240 with Lister serum, suggesting that agglutinins specific to the phases agglutinated by Lister serum are absent in Oxford serum, in so far as subculture 25 is concerned, and are partly absent in so far as subculture 28 is concerned.
3. *With R.A.M.C. serum.*
   Similar results as with Oxford serum, and with Lister serum.

From the table showing the maximum variations obtained with each serum it appears that of eleven subcultures tested respectively with R.A.M.C. serum, and with Oxford

serum, three subcultures in the first case, and four in the second case, failed to give any diagnostic readings whilst with the Lister serum two subcultures out of six failed to give reliable diagnostic readings.

Chart 6, Group N.

Attention was finally turned to *Meningococcus*, Type 1, subcultures from this "strain" being tested in parallel, *vide* Chart 6, against the four Type serums. If the morphology table is referred to, together with the serological table, it will be seen that the results obtained are suggestive. The acquisition by one "strain" of agglutinability by meningococcal serums specific to other "strains" has more than once in recent months been noted, though no satisfactory explanation has been forthcoming. The results shown in Chart 6 suggest however that the explanation lies in the existence of a morphological equivalent for each so-called strain of meningococci, and that the four meningococcal "strains" (meningococcal and parameningococcal) represent developmental phases of one strain only. That the developmental changes to be seen in meningococcal cultures are as complex as those demonstrated by warm-stage observations in Part I of this paper in the case of organisms of the enteric group I fully demonstrated in 1917, also by warm-stage observations (*vide Brit. Med. Journ.* Sept. 22, 1917). That one was perhaps then misled into regarding the unquestionable occurrence of budding, and of endofragmentation of chromatin, in the so-called giant-cells as indications that these organisms belong to the parasitic fungi does not alter the basic fact of demonstration of the fertility of the buds and of the fragments of chromatin thus produced. Nor does this affect the further fact that meningococci were shown on the warm-stage to be produced both vegetatively from pre-existing meningococci, and by chromatinolysis from giant-cells which had till then been believed to represent sterile involution forms. And the fact that in the case of the enteric group of organisms some morphological phases of development have in Part I of this paper been indisputably proved to be associated with agglutination vagaries lends collateral support to the direct evidence produced in Chart 6 that what is true of the enteric organisms is also true of the meningococcus. It appears to be a reasonable view that in the case of the enteric organisms the occurrence of budding and of branching, and of the production of gonidia, is an expression of physiological degrees respectively of plasmoptysis and plasmolysis, operating even in young standard media. And this appears also to be a reasonable explanation of the occurrence of budding in "giant" cells in meningococcal cultures under environmental conditions favourable to the induction of moderate degrees of plasmoptysis, and of the occurrence of gonidia production from chromatinolysis in the "giant" cells under conditions favourable to the induction of moderate degrees of plasmolysis. And it is noteworthy that this chromatinolysis in giant-cells in meningococcal cultures is best seen in media containing serum. It is also to be noted, emphasis being laid upon this point in the case of the enteric organisms, that these unfamiliar methods of reproduction have not been noted in cultures which appear to be exclusively composed of normal populations, the ordinary vegetative forms appearing not to depart from the ordinary methods of reproduction by equal binary fission only, even when conditions are markedly favourable for unusual types of reproduction. In the case of meningococcal cultures it appears possible that the explanation of the fact that some cultures will not easily survive, whilst others readily survive, is due to the exclusive presence of vegetative forms in the former case, and in admixture with fertile giant-forms in the second case, these being able to survive adverse circumstance in consequence of increased resistance to such circumstance, as was found to be the case with organisms of the enteric group.

CONCLUSIONS.

There appears, from these observations, to be no doubt that changes in agglutinability are often associated, in the case of the organisms examined, with the occurrence of developmental changes in such organisms, changes which have in many cases definite morphological values. It must however not be supposed that a claim is here put forward that a different morphological equivalent exists for each of the agglutination vagaries here disclosed. The subject is far too complex a one for any such claim to be made. The evidence so far produced is that of a preliminary investigation only, and is published now in the hope that other workers will devote their attention to the morphological problems dealt with. In the meanwhile an attempt is being made to correlate the results obtained with the results of testing subcultures from single cells, and to show that reliable polyvalent serums can be obtained by construing valency in terms of morphology and not in terms of "strains" unrelated to developmental phases. It will be evident that absorption tests are of little value in this work unless the morphological equivalent of each emulsion used in the preparation of a serum is first worked out.

NOTE.

As further evidence of the reliability of the macroscopic method employed throughout these observations the subjoined figures of control titration tests may be cited, the test which gave the largest error being selected. The maximum experimental error in this test is seen to be 5·3 per cent., as gauged by titration of N/20 HCl with N/20 NAOH, each tube in the total series of 10 tubes receiving measured quantities of HCl in water as follows. First filling: equal volumes of acid in all 10 tubes. Second filling: tubes 2 to 9 receive half the volume of the preceding tube, tube 1 receiving a full volume of acid, tube 10 receiving none. Third filling: each tube receives a fresh volume of acid equal to the volumes employed in the first filling. In this way the distribution of saline, serum and emulsion was reproduced as closely as possible, with the result that a severe test of accuracy was imposed with regard to the thirty measurements employed.

In 6 of the 10 tubes 2·90 c.c. of N/20 NAOH were required for neutralisation.

„ 2	„	2·70 c.c.	„	„	„
„ 1	„	2·82 c.c.	„	„	„
„ 1	„	2·85 c.c.	„	„	„

In this control test therefore we get a mean of 2·80, a mode of 2·90, and a maximum experimental error of 5·3 per cent.

It has of course long been known that inconstant serological readings may occur when observations are made with different emulsions made from one living culture at different intervals of time, using the same serum throughout. In many of the observations recorded in the text it is shown that wide

variations may occur when a series of fractions of the same emulsion from one culture are put up at the same time against different serums specific to the organism under examination, the existence of multiple strains being usually regarded as adequate explanation of such variations, the absence of considerable experimental error being assumed. The control observations given in the text and in this note show that in the case of the variations here recorded the experimental error can be excluded.

In view of the fact demonstrated in the text that wide morphological variations may occur in living fertile descendants of a single cell, some of these variations being definitely associated with wide variations in serological equivalents in terms of one serum, it would appear that the multiple strain theory is here inadequate. And this view is confirmed by the following transitions occurring within a few hours.

At 10.0 a.m., 13. ii. 18, an emulsion was made in plus 10 broth from a 13 hour 4 per cent. glucose agar culture inoculated with a pure culture of *B. typhosus*, and a fraction of the living emulsion was tested at once (*vide* A) against Lister *B. typhosus* serum: C 105: 28. xi. 17: 1/20: 1/6000.

At 2.30 p.m. on the same day a second fraction of the same living emulsion in broth was, after incubation at 37° C. for 4½ hours, tested against the same serum from the same tube (*vide* B).

Twelve hours later a third fraction of the same living emulsion in broth was, after incubation at 37° C. for 16½ hours, tested against the same serum from the same tube (*vide* C).

In each case a reading was taken after one hour at 56° C., and again after a further eight hours at 56° C. There was no difference in the two readings in any tube.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	Control	
nil	nil	nil	nil	nil	nil	nil	nil	nil	... A
C A	C A	C A	C A	C A	nil	nil	nil	nil	... B
C A	C A	C A	C A	C A	C A	C A	C A	nil	... C

C A = complete agglutination

Morphology of A ... exclusively coccoid
„ B ... mixed coccoid and bacillary
„ C ... bacillary population predominant

The transition from non-agglutinable to agglutinable, with corresponding morphological transition, is however not necessarily rapid, a more or less stable condition of non-agglutinability being sometimes met with which, in one case, lasted five days in spite of repeated subculture in broth. In such case the return of agglutinability, and of bacillary form, sometimes only takes place on subculturing to agar from broth, the inverse of the usual experience.

The work on which Part I of this paper is based was carried out by me in the laboratory of the Addington Park War Hospital. The work on which

Part II is based was undertaken in the Central Laboratory of the Kitchener Hospital, Brighton. In both cases the work was the outcome of previous work on bacterial morphology carried out by me as Director of the Constance Trotter Research Fund in a laboratory generously placed at my disposal by the Governing Body of the Lister Institute.

To my laboratory attendant, E. Hawkins, I am greatly indebted for scrupulous care in the preparation of media and other material.

NOTE EXPLAINING CHARTS.

In the following charts are given:

1. The serum dilutions employed, each dilution except the first being double that of the preceding dilution.

In all cases the end-points, denoting partial agglutination, are given, represented by the symbol +. The end-points of complete agglutinations, and symbols representing the numerous gradations between complete agglutination and that just visible with the aid of a hand-lens, are deliberately omitted so as not to confuse the issue.

In determining the end-points here recorded extreme care was taken, in each series, to study the gradations between each tube in a row of nine tubes in relation to the gradations between the tubes in all the rows. The symbol 0 represents complete absence of agglutination.

2. The numbers of each subculture, generally with the dates of subculture.

3. Keys giving the "genealogy" of the subcultures.

4. The particulars of each serum employed, as to source, date of preparation, dilution and reputed maximum titre.

5. Whenever possible, the morphological equivalent.

In Chart A the brackets opposite the numbers 3, 4, 5=6, 7, 8=11, 12=13, 14, 15, 16=1, 2, 3, 4, indicate that each group of numbers indicates identical fractions of one emulsion (*vide* NOTE).

In number 2, of date 11. I. 18, the final dilution of 10,240 was inadvertently omitted.

CHART A.

CONTROL OBSERVATIONS SHOWING RELIABILITY OF AGGLUTINATION TECHNIQUE EMPLOYED (*a*) *B. TYPHOSUS*, (*b*) *B. DYSENTERIAE*.

Morphological equivalent of each control emulsion "normal" bacilli.

B. typhosus.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	Control	Date	Number
+	+	+	+	+	+	+	+	0	10. I. 18	1
+	+	+	+	+	+	±	in last tube	0	11. I. 18	2
+	+	+	+	+	+	+	+	0	18. I. 18	3
+	+	+	+	+	+	+	+	0	,,	4
+	+	+	+	+	+	+	+	0	,,	5
+	+	+	+	+	+	+	+	0	5. II. 18	6
+	+	+	+	+	+	+	+	0	,,	7
+	+	+	+	+	+	+	+	0	,,	8
+	+	+	+	+	+	+	+	0	8. II. 18	9
+	+	+	+	+	+	+	+	0	11. II. 18	10
+	+	+	+	+	+	+	+	0	28. II. 18	11
+	+	+	+	+	+	+	+	0	,,	12
+	+	+	+	+	+	+	+	0	1. III. 18	13
+	+	+	+	+	+	+	+	0	,,	14
+	+	+	+	+	+	+	+	0	,,	15
+	+	+	+	+	+	+	+	0	,,	16

Lister *B. typhosus* serum used throughout. Dilution 1/20: titre "maximum" 1/6000: dates 11. IX. 17: 28. XI. 17, etc.

CHART A—*contd.*

1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12,800	Control	Date	Number
+	+	+	+	+	+	...	...	0	1. XI. 18	1
+	+	+	+	+	+	...	...	0	„	2
+	+	+	+	+	+	...	...	0	„	3
+	+	+	+	+	+	...	...	0	„	4

R.A.M.C. Polyvalent serum = *B. dysenteriae* ("B. Y").

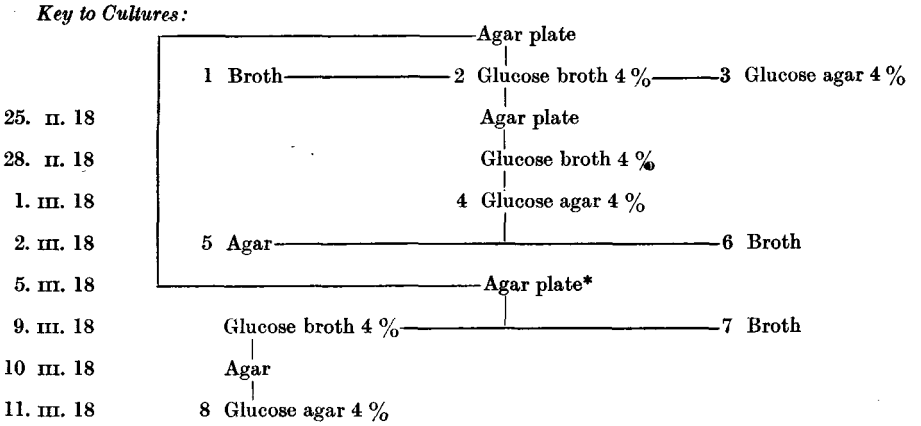
CHART 1, GROUP A.

B. typhosus, Strain Pierce *versus* Lister *B. typhosus* Serum C 105: 1/20: 1/6000: 28. XI. 17.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	Cultures	Morphology
+	+	+	+	+	+	+	+	1	Dom. pop. bacillary
+	+	+	+	+	+	+	+	2	Dom. pop. bacillary
+	+	+	+	+	+	+	0	3	Dom. pop. bacillary
0	0	0	0	0	0	0	0	4	D.P. thick-walled cocc.
+	+	+	+	+	+	+	+	5	D.P. bacillary
+	+	+	+	+	+	0	0	6	D.P. bacillary
+	+	+	+	+	+	+	+	7	D.P. bacillary
0	0	0	0	0	0	0	0	8	D.P. thick-walled cocc.

All control tubes negative.

Key to Cultures:



* This indicates a fresh plating on 5. III. 18 *direct* from original agar plate.

CHART 1, GROUP B.

B. typhosus, Strain Pierce *versus* Lister *B. typhosus* Serum C 105: 1/20: 1/6000: 18. I. 18.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	1/40,960	Cult.	Morphol.
0	0	0	0	0	0	0	0	0	0	2	Coccoidal
+	+	+	0	0	0	0	0	0	0	1	Coccoidal
+	+	+	+	+	0	0	0	0	0	3	Cocco-bacill.
+	+	+	+	+	+	+	+	0	0	A	Bacillary
+	+	+	+	+	+	+	+	+	0	5	Bacillary
+	+	+	+	+	+	+	+	+	+	4	Bacillary
+	+	+	+	+	+	+	+	+	+	6	Bacillary

All control tubes negative.

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Key to Cultures (Chart 1, Group B):

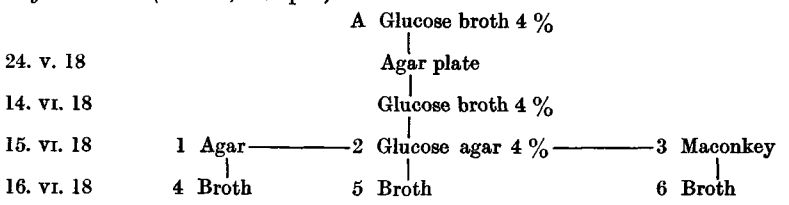

CHART 1, GROUP C.

B. typhosus, Strain Pierce *versus* Lister *B. typhosus* Serum C 105: 1/20: 1/6000: 6. vi. 18.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	1/40,960	Cult.	Morphol.
+	+	0	0	0	0	0	0	0	0	1	Coccoidal
+	+	0	0	0	0	0	0	0	0	2	Coccoidal
+	+	+	+	+	+	+	+	+	+	3	Bacillary
0	0	0	0	0	0	0	0	0	0	4	Coccoidal
0	0	0	0	0	0	0	0	0	0	5	Coccoidal

All control tubes negative.

Key to Cultures:

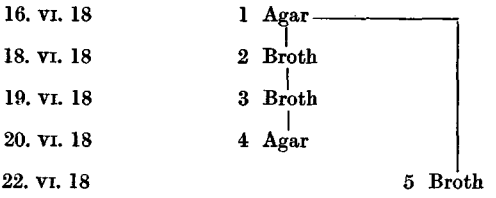

CHART 1, GROUP D.

B. typhosus, Strain Pierce *versus* Lister *B. typhosus* Serum and R.A.M.C. *B. typhosus* Serum.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	Cultures	Serum	Colony	Cont.
+	+	+	0	0	0	0	0	0	7	Lister	A 1	0
+	+	+	+	+	+	+	0	0	5			0
+	+	+	+	+	+	+	+	0	3			0
+	+	+	+	+	+	+	+	0	1, 2, 4, 6, 8, 9, 10			0
+	+	+	0	0	0	0	0	0	7, 9	R.A.M.C.	A 2	0
+	+	+	+	0	0	0	0	0	1			0
+	+	+	+	+	0	0	0	0	2, 5, 6,			0
+	+	+	+	+	+	0	0	0	4			0
+	+	+	+	+	+	+	0	0	3, 8			0
+	+	+	+	+	+	+	+	0	10			0
+	+	0	0	0	0	0	0	0	7	Lister	B 1	0
+	+	+	+	+	+	+	+	0	1, 2, 8, 9, 10			0
+	+	0	0	0	0	0	0	0	7, 9	R.A.M.C.		0
+	+	+	+	0	0	0	0	0	1			0
+	+	+	+	+	0	0	0	0	2, 10			0
+	+	+	+	+	+	0	0	0	8			0

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Chart 1, Group D—*contd.*

Maximum Variations irrespective of which Serum is employed.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	Cultures	Colony
+	+	+	...	...	...	...	...	...	7, 9	A 1, A 2
+	+	+	+	...	...	...	...	...	1	
+	+	+	+	+	...	...	...	...	2, 5, 6	
+	+	+	+	+	+	...	...	...	4	
+	+	+	+	+	+	+	...	...	5, 3, 8	
+	+	+	+	+	+	+	+	...	3	
+	+	+	+	+	+	+	+	+	1, 2, 4, 6, 8, 9, 10	
+	+	...	...	...	...	...	...	...	7, 9	B 1, B 2
+	+	+	+	...	...	...	...	...	1	
+	+	+	+	+	...	...	...	...	2, 10	
+	+	+	+	+	+	...	...	...	8	
+	+	+	+	+	+	+	+	+	1, 2, 8, 9, 10	

Key to Agglutinations:

A 1. *B. typhosus* Colony 1 *v.* Lister serum C 105 : 1/20 : 1/6000 : 23. VIII. 18.
A 2. *B. typhosus* Colony 1 *v.* R.A.M.C. serum : 1/20 : 1/7000 : 18. IX. 18.
B 1. *B. typhosus* Colony 1 *v.* Lister serum C 105 : 1/20 : 1/6000 : 23. VIII. 18.
B 2. *B. typhosus* Colony 1 *v.* R.A.M.C. serum : 1/20 : 1/7000 : 18. IX. 18.

Key to Cultures:

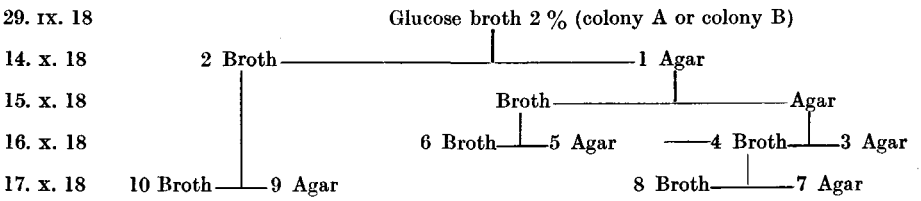

Chart 1, Group E.

B. typhosus, Strain Pierce *versus* R.A.M.C. *B. typhosus* Serum and Oxford *B. typhosus* Serum.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	1/40,960	Cult.	Serum	Cont.
+	+	+	+	+	+	+	+	+	+	1	Oxford	0
+	+	+	+	+	+	+	+	+	+	2		0
+	+	+	+	+	+	+	+	+	+	3		0
+	+	+	0	0	0	0	0	0	0	1	R.A.M.C.	0
+	+	+	+	0	0	0	0	0	0	2		0
+	+	+	+	+	+	0	0	0	0	3		0

Key to Cultures:

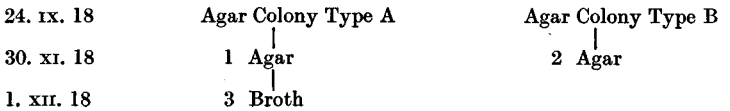

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CHART 2, GROUP F.

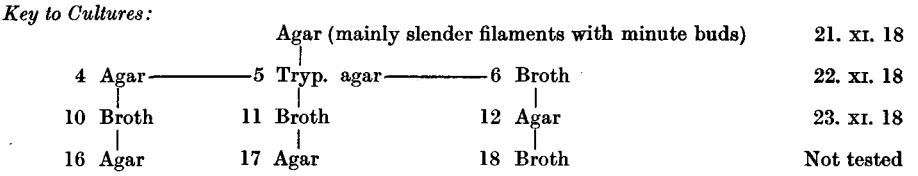
B. typhosus, Oxford Strain Edwin *versus* Oxford Serum, Lister Serum and R.A.M.C. Serum.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	1/40,960	Cultures	Serum
+	+	+	+	+	0	0	0	0	0	11	Oxford
+	+	+	+	+	+	0	0	0	0	5	
+	+	+	+	+	+	+	0	0	0	4, 6, 10	
+	+	+	+	+	+	+	+	0	0	12	
+	+	+	+	+	+	+	+	0	0	5, 6, 11	Lister
+	+	+	+	+	+	+	+	+	0	4, 12	
+	+	+	+	+	+	+	+	+	0	10	
+	+	+	+	0	0	0	0	0	0	12, 6	R.A.M.C.
+	+	+	+	+	0	0	0	0	0	11, 5	
+	+	+	+	+	+	0	0	0	0	10	
+	+	+	+	+	+	+	0	0	0	4	

MAXIMUM VARIATIONS IRRESPECTIVE OF WHICH SERUM IS EMPLOYED.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	1/40,960	Cultures
+	+	+	+	...	...	...	...	...	...	12, 6
+	+	+	+	+	...	...	...	...	...	11, 5
+	+	+	+	+	+	...	...	...	...	10, 5
+	+	+	+	+	+	+	...	...	...	4, 6, 10
+	+	+	+	+	+	+	+	...	...	12, 5, 6, 11
+	+	+	+	+	+	+	+	+	...	12, 4
+	+	+	+	+	+	+	+	+	+	10

Key to Cultures:



All control tubes negative.

Stock Oxford culture despatched 20. XI. 18 : received 21. XI. 18.

CHART 3, GROUP G.

B. dysenteriae: Lister Stock Flexner Culture and Lister Stock Y Culture *versus* Lister Stock Serums B. Flexner and B. Y.

E.A.M.	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	Control	Cultures	Experiment
10	0	0	0	0	0	0	0	0	2, 4	A
	+	+	0	0	0	0	0	0	1	
	+	+	+	0	0	0	0	0	3	
13	0	0	0	0	0	0	0	0	1, 4	B
	+	+	0	0	0	0	0	0	3	
	+	+	+	+	+	0	0	0	2	
16	0	0	0	0	0	0	0	0	2	C
	+	+	0	0	0	0	0	0	4	
	+	+	+	0	0	0	0	0	3	
	+	+	+	+	0	0	0	0	1	
13	0	0	0	0	0	0	0	0	1	D
	+	+	+	0	0	0	0	0	3, 4	
	+	+	+	+	0	0	0	0	2	

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Chart 3, Group G—*contd.*

Morphology of Lister B. Flexner Culture (Stock).

Dominant population "normal" bacilli.

Morphology of B. Flexner Subcultures.

1. Slender bacilli, and many bacilli showing Artaud's nodes.
2. "Normal" bacilli.
3. Short fat bacilli, uniformly stained.
4. Small coccoids, large diplococcoids, large ovoids.

Morphology of Lister B. Y Culture (Stock).

Dominant population very minute cocco bacilli.

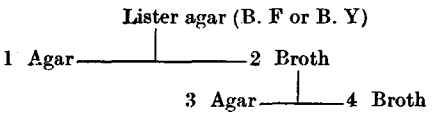
Morphology of B. Y Subcultures.

1. Minute coccoids and diplococcoids.
2. ? "Normal" bacilli.
3. Minute coccoids and minute bacilli.
4. Minute coccoids and diplococcoids : no bacilli.

B. dysenteriae.

Key to Cultures:

Key to Chart 3, Group G.



Key to Agglutinations:

A. Lister B. Flexner Culture *v.* Lister B. Flexner serum C 94 : 1/20 : 1/3000 : 27. III. 18.
B. Lister B. Flexner Culture *v.* Lister B. Y serum C 137 : 1/10 : 1/1500 : 27. III. 18.
C. Lister B. Y Culture *v.* Lister B. Flexner serum C 94 : 1/20 : 1/3000 : 27. III. 18.
D. Lister B. Y Culture *v.* Lister B. Y serum C 137 : 1/10 : 1/1500 : 27. III. 18.

Chart 3, Group H.

B. dysenteriae: R.A.M.C. B. Flexner Stock Culture and R.A.M.C. B. Y Stock Culture *versus* R.A.M.C. Serums B. Flexner and B. Y.

E.A.M.	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	Control	Cultures	Experiment
15	+	+	0	0	0	0	0	0	2	A
	+	+	+	+	0	0	0	0	5, 6, 4	
	+	+	+	+	+	+	0	0	1, 3	
10	+	+	0	0	0	0	0	0	4	B
	+	+	+	0	0	0	0	0	5, 6	
	+	+	+	+	0	0	0	0	1, 2, 3	
6	0	0	0	0	0	0	0	0	2, 5, 6, 4, 3	C
	+	+	+	+	0	0	0	0	1	
17	+	+	0	0	0	0	0	0	6	D
	+	+	+	0	0	0	0	0	3	
	+	+	+	+	0	0	0	0	2, 4	
	+	+	+	+	+	0	0	0	1, 5	

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CHART 3, GROUP H—*contd.*

MORPHOLOGY OF B. FLEXNER CULTURES 3 TO 6.

3. Short bacilli.
4. "Normal" bacilli.
5. Coccoids and bacilli.
6. Short bacilli.

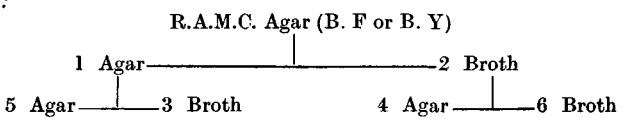
MORPHOLOGY OF B. Y CULTURES 3 TO 6.

3. Bacilli greatly varying in size: large spheroidal forms.
4. Large bacilli: numerous minute bacteroids.
5. ? "Normal" bacilli.
6. Large broad bacillary forms.

B. dysenteriae.

Key to Cultures:

KEY TO CHART 3, GROUP H.



Key to Agglutinations:

A. R.A.M.C. B. Flexner Culture Ledingham 24. VI. 18 *v.* R.A.M.C. Flexner Ledingham serum F 7 : 1/20 : 1/8000 : 27. VI. 18.

B. R.A.M.C. B. Flexner Culture Ledingham 24. VI. 18 *v.* R.A.M.C. Y Ledingham serum: 1/7000 : 27. VI. 18.

C. R.A.M.C. Y Culture Ledingham 24. VI. 18 *v.* R.A.M.C. Flexner Ledingham serum F 7 : 1/20 : 1/8000 : 27. VI. 18.

D. R.A.M.C. Y Culture Ledingham 24. VI. 18 *v.* R.A.M.C. Y Ledingham serum : 1/7000 : 27. VI. 18.

CHART 3, GROUP I.

B. dysenteriae: R.A.M.C. B. Flexner Culture Ledingham *versus* R.A.M.C. B. Flexner, Lister B. Flexner and Lister B. FY Serums.

E.A.M.	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	Cultures	Serums	Exp.
15	+	+	0	0	0	0	0	0	0	2	R.A.M.C.	A
	+	+	+	+	0	0	0	0	0	5, 6, 4		
	+	+	+	+	+	+	0	0	0	1, 3	culture	24. VI. 18
42	+	+	0	0	0	0	0	0	0	4	R.A.M.C.	B
	+	+	+	+	+	+	0	0	0	5		
	+	+	+	+	+	+	+	+	0	1, 3	culture	2. X. 18
	+	+	+	+	+	+	+	+	+	2, 6		
5	0	0	0	0	0	0	0	0	0	1, 4, 5	Lister F C 94	
	+	+	0	0	0	0	0	0	0	3, 6		
	+	+	+	0	0	0	0	0	0	2		
32	+	+	0	0	0	0	0	0	0	4	Lister F Y C 70	
	+	+	+	+	0	0	0	0	0	5		
	+	+	+	+	+	0	0	0	0	1		
	+	+	+	+	+	+	...	...	...	3, 6		
	+	+	+	+	+	+	+	0	0	2		

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Chart 3, Group I—*contd.*

Maximum Variations obtained irrespective of Serums employed.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480
0	...	...	...	...	...	...	...	...
+	+	...	...	...	...	...	...	...
+	+	+	...	...	...	...	...	...
+	+	+	+	...	...	...	...	...
+	+	+	+	+	...	...	...	...
+	+	+	+	+	+	...	...	...
+	+	+	+	+	+	+	...	...
+	+	+	+	+	+	+	+	...
+	+	+	+	+	+	+	+	+

B. dysenteriae.

Key to Chart 3, Group I.

Key to Cultures:

R.A.M.C. B. Flexner Stock Culture Ledingham — 24. vi. 18

Agar — 2. x. 18

1 Agar — 2 Broth — 3. x. 18

5 Agar — 3 Broth; 4 Agar — 6 Broth — 4. x. 18

Key to Agglutinations:

A. R.A.M.C. B. Flexner Culture Ledingham 24. vi. 18 *v.* R.A.M.C. B. Flexner Ledingham serum F 7: 1/20: 27. vi. 18.

B. R.A.M.C. B. Flexner Culture Ledingham 2. x. 18 *v.* R.A.M.C. B. Flexner Ledingham serum F 7: 1/20: 27. vi. 18.

C. R.A.M.C. B. Flexner Culture Ledingham 2. x. 18 *v.* Lister B. Flexner serum C 94: 1/20: 1/3000: 27. iii. 18.

D. R.A.M.C. B. Flexner Culture Ledingham 2. x. 18 *v.* Lister B. FY serum C 70: 1/10: 1/2000: 12. i. 18(?).

Chart 3, Group K 1.

B. dysenteriae: R.A.M.C. B. Flexner Culture Ledingham *versus* R.A.M.C. Serums FY 3, Y 55, F 8 A and Lister Serum FY C 137.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	Serum	Plate	Medium
+	+	0	0	0	0	0	0	0	A	G ½ colony	Agar
+	+	+	0	0	0	0	0	0	B, C	,,	,,
+	+	+	+	+	+	+	+	+	D	,,	,,
+	+	½	+	0	0	0	0	0	B	G ½ colony	Broth
+	+	+	+	+	+	0	0	0	C	,,	,,
+	+	+	+	+	+	+	0	0	A	,,	,,
+	+	+	+	+	+	+	+	+	D	,,	,,
+	0	0	0	0	0	0	0	0	B, D	H ½ colony	Agar
+	+	+	0	0	0	0	0	0	A, C	,,	,,
+	+	+	+	0	0	0	0	0	A, B	H ½ colony	Broth
+	+	+	+	+	0	0	0	0	C	,,	,,
+	+	+	+	+	+	+	0	0	D	,,	,,

(A) R.A.M.C. serum F Y 3; (B) R.A.M.C. serum Y 55; (C) Lister serum FY C 137; (D) R.A.M.C. serum F 8 A.

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CHART 3, GROUP K 1—*contd.*

MAXIMUM VARIATION IRRESPECTIVE OF SERUM.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240
+	...	...	...	...	...	...	...
+	+	...	...	...	...	...	...
+	+	+	...	...	...	...	...
+	+	+	+	...	...	...	...
+	+	+	+	+	...	...	...
+	+	+	+	+	+	...	...
+	+	+	+	+	+	+	...
+	+	+	+	+	+	+	+

CHART 3, GROUP K 2.

B. dysenteriae: R.A.M.C. B. Flexner Culture Ledingham *versus* R.A.M.C. Serums FY 3, Y 55, F 8 A and Lister Serum C 94.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	Serum	Plate	Medium
0	0	0	0	0	0	0	0	0	A	D ½ colony	Agar
0	0	0	0	0	0	0	0	0	A	D ½ „	Broth
0	0	0	0	0	0	0	0	0	A	C ½ „	Agar
+	+	0	0	0	0	0	0	0	A	C ½ „	Broth
+	+	+	0	0	0	0	0	0	B	D ½ „	Agar
+	+	+	0	0	0	0	0	0	B	C ½ „	Agar
+	+	+	+	0	0	0	0	0	B	D ½ „	Broth
+	+	+	+	0	0	0	0	0	B	C ½ „	Broth
0	0	0	0	0	0	0	0	0	C	D ½ „	Broth
+	+	+	0	0	0	0	0	0	C	D ½ „	Agar
+	+	+	0	0	0	0	0	0	C	C ½ „	Agar
+	+	+	+	+	0	0	0	0	C	C ½ „	Broth
+	+	+	+	0	0	0	0	0	D	D ½ „	Agar
+	+	+	+	+	+	+	+	0	D	D ½ „	Broth
+	+	+	+	+	+	+	+	0	D	C ½ „	Agar
+	+	+	+	+	+	+	+	0	D	C ½ „	Broth

(A) Lister B. Flexner serum C 94; (B) R.A.M.C. serum Y 55; (C) R.A.M.C. serum FY 3; (D) R.A.M.C. serum F 8 A.

MAXIMUM VARIATION IRRESPECTIVE OF SERUM.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	Serum
0	...	...	...	...	...	...	...	...	
+	+	...	...	...	...	...	...	...	
+	+	+	...	...	...	...	...	...	
+	+	+	+	...	...	...	...	...	
+	+	+	+	+	...	...	...	...	
+	+	+	+	+	+	+	+	...	

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Chart 3, Group K 3.

B. dysenteriae: R.A.M.C. B. Flexner Culture Ledingham *versus* R.A.M.C. Serums FY 3, Y 55, F 8 A and Lister Serum C 94.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	Serum	Plate	Medium
0	0	0	0	0	0	0	0	0	A	E ½ colony	Agar
+	+	0	0	0	0	0	0	0	B	,,	,,
+	+	+	0	0	0	0	0	0	C	,,	,,
+	+	+	+	+	+	+	+	+	D	,,	,,
+	0	0	0	0	0	0	0	0	D	E ½ colony	Broth
+	+	+	0	0	0	0	0	0	B	,,	,,
+	+	+	+	0	0	0	0	0	C	,,	,,
+	+	+	+	+	0	0	0	0	A	,,	,,
+	0	0	0	0	0	0	0	0	A	F ½ colony	Agar
+	+	+	0	0	0	0	0	0	C	,,	,,
+	+	+	+	0	0	0	0	0	B	,,	,,
+	+	+	+	+	+	+	+	+	D	,,	,,
+	0	0	0	0	0	0	0	0	A	F ½ colony	Broth
+	+	+	0	0	0	0	0	0	B	,,	,,
+	+	+	+	+	0	0	0	0	C	,,	,,
+	+	+	+	+	+	+	+	+	D	,,	,,

(A) Lister B. Flexner serum C 94; (B) R.A.M.C. serum B. Y 55; (C) R.A.M.C. serum B. FY 3; (D) R.A.M.C. serum F 8 A.

Maximum Variation irrespective of Serum.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480
+	...	...	...	...	...	...	...	...
+	+	...	...	...	...	...	...	...
+	+	+	...	...	...	...	...	...
+	+	+	+	...	...	...	...	...
+	+	+	+	+	...	...	...	...
+	+	+	+	+	+	+	+	+

Chart 4, Group L 1.

B. dysenteriae: B. Y Kitchener Strain (B) *versus* R.A.M.C. Polyvalent Serum, FY 3, Y 55, Y 4, and Lister FY C 137 Serum.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	Serum	Cultures
+	+	0	0	0	0	0	0	0	R.A.M.C. FY 3	18, 19
+	+	+	0	0	0	0	0	0		12
+	+	+	+	0	0	0	0	0		10, 13
+	+	+	+	+	0	0	0	0		11
+	+	+	0	0	0	0	0	0	Lister FY C 137	8, 9, 12
+	+	+	+	0	0	0	0	0		10
+	+	+	+	+	0	0	0	0		11
+	+	+	+	+	+	0	0	0		13
+	+	0	0	0	0	0	0	0	R.A.M.C. Polyvalent	20, 21
+	+	+	+	+	0	0	0	0		14
+	+	+	+	+	+	0	0	0		16
+	+	+	+	+	+	0	0	0		15
+	+	+	+	+	+	0	0	0		17

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CHART 4, GROUP L 1—*contd.*

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	Serum	Cultures
+	+	+	0	0	0	0	0	0		13
+	+	+	+	+	0	0	0	0		8, 9
+	+	+	+	+	+	0	0	0	R.A.M.C.	1, 7, 12
+	+	+	+	+	+	+	0	0	Y 55	3, 6
+	+	+	+	+	+	+	+	...		10, 11
+	+	+	+	+	+	+	+	+		2, 4, 5
+	+	+	0	0	0	0	0	0	R.A.M.C. Y 4	20, 21

MAXIMUM VARIATIONS IRRESPECTIVE OF SERUMS.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480
+	+	...	...	...	...	...	...	...
+	+	+	...	...	...	...	...	...
+	+	+	+	...	...	...	...	...
+	+	+	+	+	...	...	...	...
+	+	+	+	+	+	...	...	...
+	+	+	+	+	+	+	...	...
+	+	+	+	+	+	+	+	...
+	+	+	+	+	+	+	+	+

Key to Cultures:

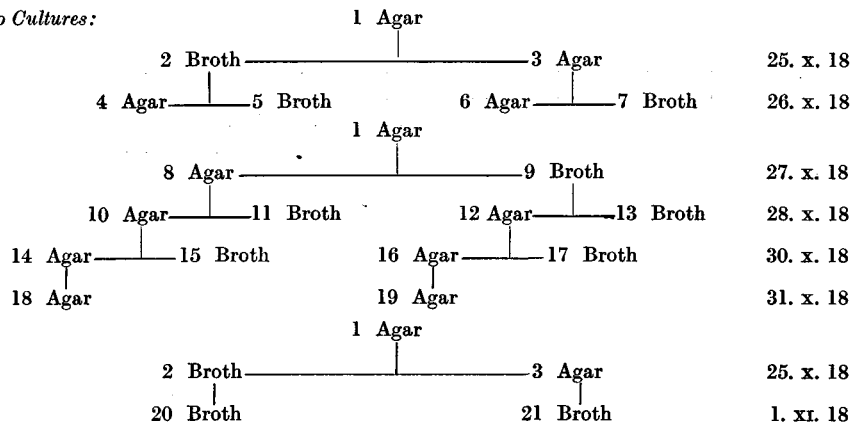
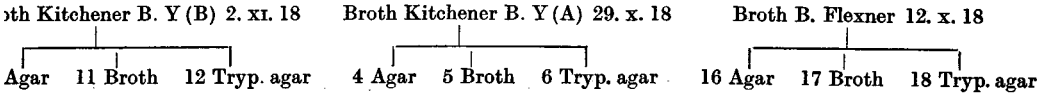


CHART 4, GROUP L 2.

B. dysenteriae: R.A.M.C. Polyvalent Flexner Y Serum D 1: 1/100: 1/5000: 15. XI. 8 *versus* two Y strains and one F strain.

100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12,800	1/25,600	Cultures	Medium	Strain
+	+	+	+	+	+	0	0	0	6	Tryp. Agar	Kitchener Y (A)
+	+	+	+	+	+	+	0	0	5	Broth	
+	+	+	+	+	+	+	+	0	4	Agar	
+	+	+	+	+	+	0	0	0	10	Agar	Kitchener Y (B)
+	+	+	+	+	+	+	0	0	12	Tryp. Agar	
+	+	+	+	+	+	+	+	+	11	Broth	
+	+	+	+	0	0	0	0	0	16	Agar	R.A.M.C. B F Led.
+	+	+	+	+	0	0	0	0	18	Tryp. Agar	
+	+	+	+	+	+	0	0	0	17	Broth	

y to Cultures:



Date of all subcultures 23. XI. 18.

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Chart 5, Group M.

B. dysenteriae: *B. Shiga-Kruse* Culture Oxford *versus* Oxford Shiga Serum, R.A.M.C. Shiga Serum and Lister Shiga Serum.

Maximum Variations with each Serum.

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	1/40,960	Serum: cultures
										R.A.M.C.
+	...	...	...	...	...	...	...	...	...	25, 27, 28
+	+	+	+	...	...	...	...	...	...	19
+	+	+	+	+	...	...	...	...	...	14
+	+	+	+	+	+	...	...	...	...	13, 21
+	+	+	+	+	+	+	...	...	...	20, 31, 32
+	+	+	+	+	+	+	+	+	...	15
										Oxford
+	...	...	...	...	...	...	...	...	...	19, 20, 21, 25
+	+	+	+	+	+	...	...	...	...	28
+	+	+	+	+	+	+	+	+	...	31, 32
+	+	+	+	+	+	+	+	+	+	13, 14, 15, 27
										Lister
+	...	...	...	...	...	...	...	...	...	14
+	+	...	...	...	...	...	...	...	...	13
+	+	+	...	...	...	...	...	...	...	15
+	+	+	+	+	+	+	+	...	...	25, 28
+	+	+	+	+	+	+	+	+	...	27

Using the Oxford serum 3 subcultures out of 11 gave readings below the diagnostic titre.
„ R.A.M.C. „ 4 „ 11 „ „ „
„ Lister „ 2 „ 6 „ „ „

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	1/40,960	Cultures	Serum
+	+	+	+	+	0	0	0	0	0	14	R.A.M.C.
+	+	+	+	+	+	0	0	0	0	13	„
+	+	+	+	+	+	+	+	+	0	15	„
+	+	+	+	+	+	+	+	+	+	13	Oxford
+	+	+	+	+	+	+	+	+	+	14	„
+	+	+	+	+	+	+	+	+	+	15	„
+	+	0	0	0	0	0	0	0	0	13	Lister
+	0	0	0	0	0	0	0	0	0	14	„
+	+	+	0	0	0	0	0	0	0	15	„
+	+	+	+	0	0	0	0	0	0	19	R.A.M.C
+	+	+	+	+	+	0	0	0	0	21	„
+	+	+	+	+	+	+	0	0	0	20	„
+	0	0	0	0	0	0	0	0	0	19	Oxford
+	0	0	0	0	0	0	0	0	0	20	„
+	0	0	0	0	0	0	0	0	0	21	„
+	0	0	0	0	0	0	0	0	0	25	R.A.M.C.
+	0	0	0	0	0	0	0	0	0	27	„
+	0	0	0	0	0	0	0	0	0	28	„
0	0	0	0	0	0	0	0	0	0	25	Oxford
+	+	+	+	+	+	0	0	0	0	28	„
+	+	+	+	+	+	+	+	+	0	27	„

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CHART 5, GROUP M—*contd.*

1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10,240	1/20,480	1/40,960	Cultures	Serum
+	+	+	+	+	+	+	+	0	0	25	Lister
+	+	+	+	+	+	+	+	0	0	28	
+	+	+	+	+	+	+	+	+	0	27	„
+	+	+	+	+	+	+	0	0	0	31	R.A.M.C
+	+	+	+	+	+	+	0	0	0	32	„
+	+	+	+	+	+	+	+	+	0	31	Oxford
+	+	+	+	+	+	+	+	+	0	32	„

Key to Cultures:

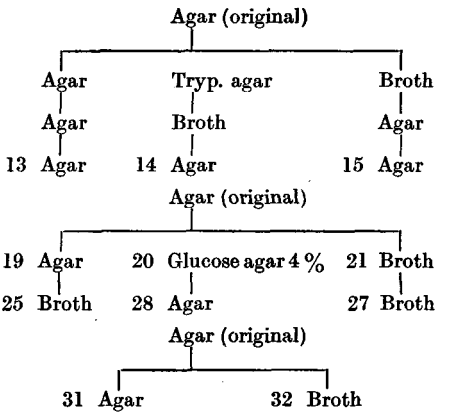

CHART 6, GROUP N.

Meningococcus Type 1 *versus* R.A.M.C. Serums Types 1, 2, 3, 4.

0	1/100	1/200	1/400	1/800	1/1600	Control	Cultures	Serums	Serums
+	0	0	0	0	0	0	14, 21	(J 4 E.T. 1/1000)	Type 4
+	+	0	0	0	0	0	1, 15, 16, 17, 30 A	—	—
+	+	+	0	0	0	0	3, 7, 8, 26, 31 A, 31 C, 34	—	—
+	+	+	+	0	0	0	23	—	—
+	+	+	+	+	0	0	4	—	—
+	0	0	0	0	0	0	1, 3, 4, 21	(J 3 E.T. 1/800)	Type 3
+	+	0	0	0	0	0	30 A, 34	—	—
+	+	+	0	0	0	0	7, 8, 14, 15, 26, 31 A	—	—
+	+	+	+	0	0	0	16, 17, 31 C	—	—
+	+	+	+	+	0	0	23	—	—
+	+	0	0	0	0	0	1, 3, 4, 23, 26, 30 A	(N 2 E.T. 1/800)	Type 2
+	+	+	0	0	0	0	8, 14, 15, 17, 21, 31 A, 34	—	—
+	+	+	+	0	0	0	7, 16		
+	+	+	+	+	0	0	31 C	—	—
+	+	+	0	0	0	0	34, 31 C	(F 1 E.T. 1/1200)	Type 1
+	+	+	+	0	0	0	4, 7, 16, 21, 26, 30 A, 1	—	—
+	+	+	+	+	0	0	3, 8, 14, 15, 17, 23, 31 A	—	—

E.A.M. with serum type 4 17·2
„ „ „ 3 17·10
„ „ „ 2 19·12
„ „ „ 1 29·12

Cultures 4, 23, 31 C agglutinate with serums 2, 3 or 4 respectively as well as does any single culture with serum 1: 1/800.

Cultures 23, 16, 17, 31 C, 7 agglutinate with serums 2, 3 or 4 respectively as well as do cultures 4, 7, 16, 21, 26, 30 A, 1 with serum 1: 1/400.

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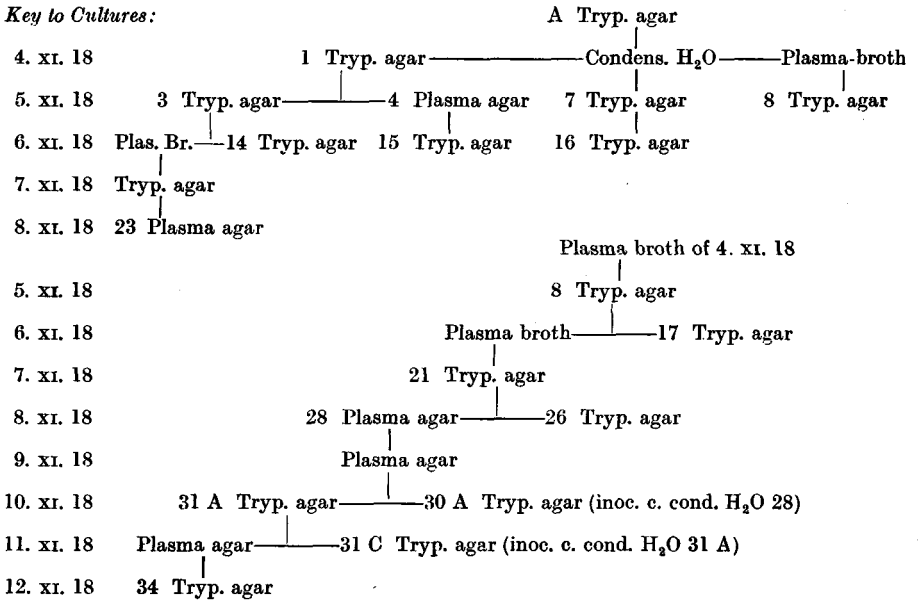
CHART 6, GROUP N—*contd.*



Meningococcus Type 1: *Morphological Equivalents of Subcultures.*

A. Chiefly small equally dividing meningococci: several deeply staining "giant" forms, some dividing equally, others unequally (budding), others showing central clear space.

1. Normal meningococci, equally dividing: uniform in size and staining: no "giant" forms seen.

3. Large non-dividing forms: three and four times the diameter of meningococci.

4. Normal meningococci mixed with minute forms, some of which are about 0·2 microns in diameter: a few small deeply-staining non-dividing forms.

7. Normal dividing meningococci, some very minute: no "giant" forms seen.

8. Normal dividing meningococci, some very minute: a few "giant" forms, numerous Gram negative minute bacilli: organisms stain uniformly.

14. *Vide* 15.

15. Medium sized "giant" forms, only a few showing signs of division: several small bacillary forms (Arkwright, d'Este Emery and others).

16. Dominant population lightly-staining "giant" forms, many undergoing unequal fission: occasional small bacillary forms.

23. Large organisms staining uniformly: many staining deeply with double contour outline, suggesting thick walls.

21. Large deeply-staining organisms, occurring singly, in short chains and in clusters. No evidence of division. Some have clear centres, in others the clear space is placed laterally, perhaps a profile effect. Population as a whole homogeneous in size. In some cases the clear centre occupies the greater part of the cell. The film does not in the least degree represent a meningococcal preparation.

26. Normal dividing meningococci, mixed in some parts of the film with large numbers of small bacilli, which are in many cases throwing off Gram negative buds, indistinguishable from meningococci: several bacilli are undergoing spherical segmentation in the terminal part of the bacillary axis.

17. No meningococci seen: population appearing entirely to consist of "giant" cells of all sizes, mainly undergoing unequal binary fission.

31 A. "Giant" cells of all sizes, from very small to large: some staining deeply, with thickened walls. A few equally dividing meningococci seen, and a few minute bacillary forms, some being of the wisp type.

30 A. Normal equally dividing meningococci, and a few large "giant" forms, with deeply-staining outline, and clear centres: numerous chains of 3, 4, 5 or 6 organisms: one chain of 8 organisms.

34. Extremely minute single and dividing cocci, some measuring about 0·1 to 0·2 microns in diameter. Some of the larger forms, from 0·4 upwards, stain more deeply than the smaller forms, some of which occur in short chains.

(Numbers refer to films and not to cultures.)

ROUGH SUMMARY OF RESULTS.

Although true meningococci are represented in serum 1, as shown by the high agglutination figure reached with serum 1 in the case of subculture 8, it appears that the antigenic value of strain 1, as represented in serum 1, finds its chief expression in "giant" forms, the morphological equivalent of films 3, 14, 17, 23, 31 A, from the subcultures which give the highest readings with serum 1 (except 8), being mainly that of "giant" forms in various stages of development. As I showed in 1916 "giant" forms may be either large, small or intermediate in size, and can at once be distinguished from true meningococci on the warm-stage by the fact that they often multiply by unequal binary fission, and by the further fact that they often undergo in addition endo-chromatinolysis, giving rise directly to meningococci which then divide in the ordinary vegetative manner. These fertile "giant" forms are readily distinguishable from forms undergoing genuine involutionary changes, both by the sterility of the latter when observed on the warm stage and by their feebly-staining properties.

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EXPLANATION OF PLATES IV—VII.

PLATE IV.

B. typhosus subcultured on + 10 agar from 4 % glucose broth. Composite selected fields. Agar cultures 1 to 3 hours, glucose cultures 12 hours.

1. Thick-walled cells (*a*) resting, (*b*) germinating. 2. Bacteroids living. 3. Sporangoids living. 4. Chlamydosporoids living. 5. Oidioids living. 6. Gonidioids living. 7. Coccoids stained. 8. Giant-cells stained. 9. Flagella unstained. 10. Mixed field living.

Series 1—10, × 1500.

PLATE V.

B. typhosus: warm-stage observations on + 10 agar from 4 % glucose broth.

Series A—D, × 1500.

PLATE VI.

B. typhosus: warm-stage observations on + 10 agar from 4 % glucose broth.

Series E—J, × 3000.

PLATE VII.

B. typhosus. Developmental phases associated with loss of agglutinability (photographs by F. Martin Duncan, × 1000).

1, 5. Thin-walled coccoids (agglutination nil), Lister serum.
4, 8. Thick-walled coccoids and normal bacilli (agglutination 1/1280), Lister serum.
2, 3, 6. Thick-walled coccoids (agglutination nil), Lister serum.
7. Thick-walled coccoids and baccilli (agglutination 1/160), Lister serum.
Pure bacillary subcultures from these coccoids agglutinated to 1/40,960, Lister serum. For warm-stage observations of growth from coccoid to bacillus *v.* Plate VI, G.

TIME-TABLE OF PLATES V AND VI.

A. 4.55, 5.10, 5.22, 5.35, 5.45, 6.0, 6.12, 6.50 p.m.
B. 7.35, 7.50, 8.15, 8.40, 9.0, 9.20, 9.40, 10.0 p.m.
C. 8.40, 9.10, 9.40, 10.10, 10.30 p.m.
D. 7.0, 7.40, 8.10, 9.0, 9.15, 9.50, 10.15 p.m.
E, F. 10.0, 10.5, 10.10, 10.20, 10.25, 10.40, 11.10 to 12.0 p.m.
I. 7.30, 8.0, 8.35, 9.0 p.m.
J. 6.25, 6.35, 6.44, 6.48 p.m.

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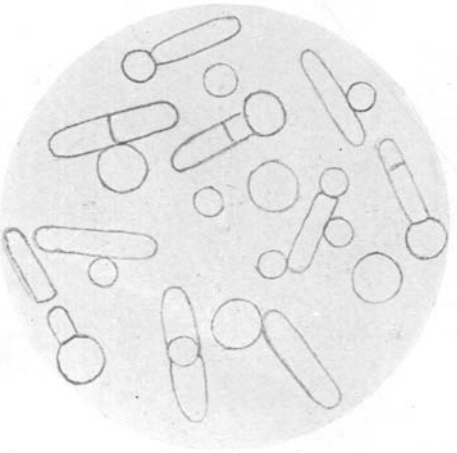

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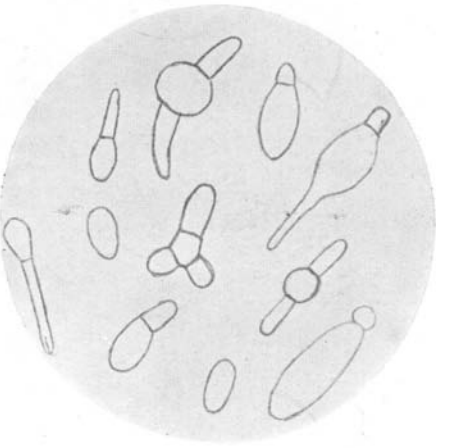

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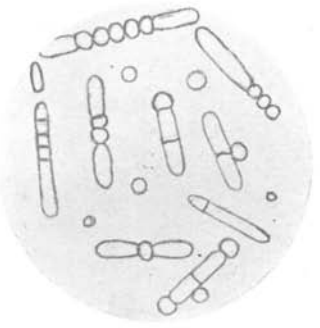
PLATE IV



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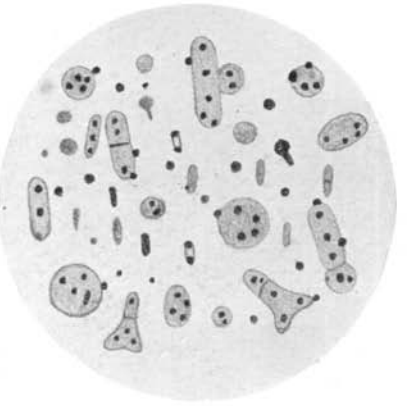

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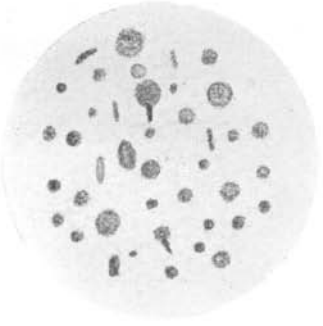

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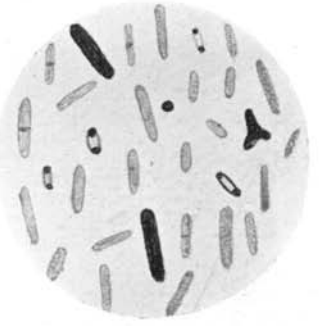

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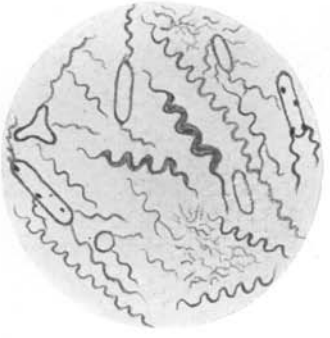

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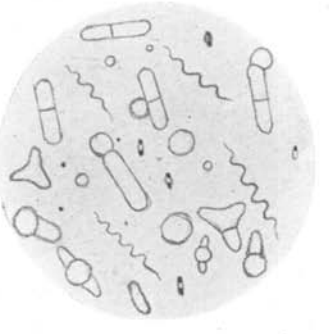

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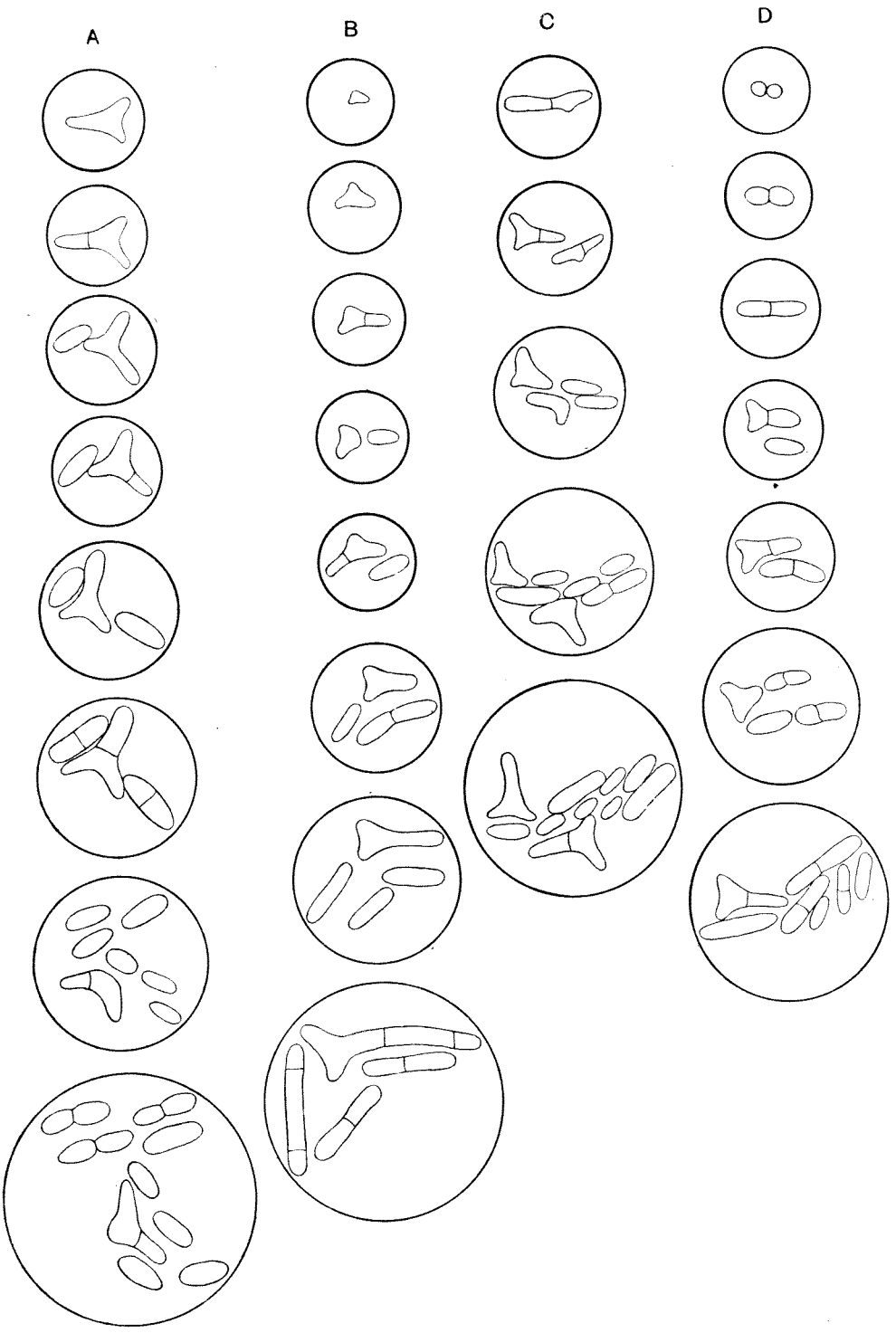

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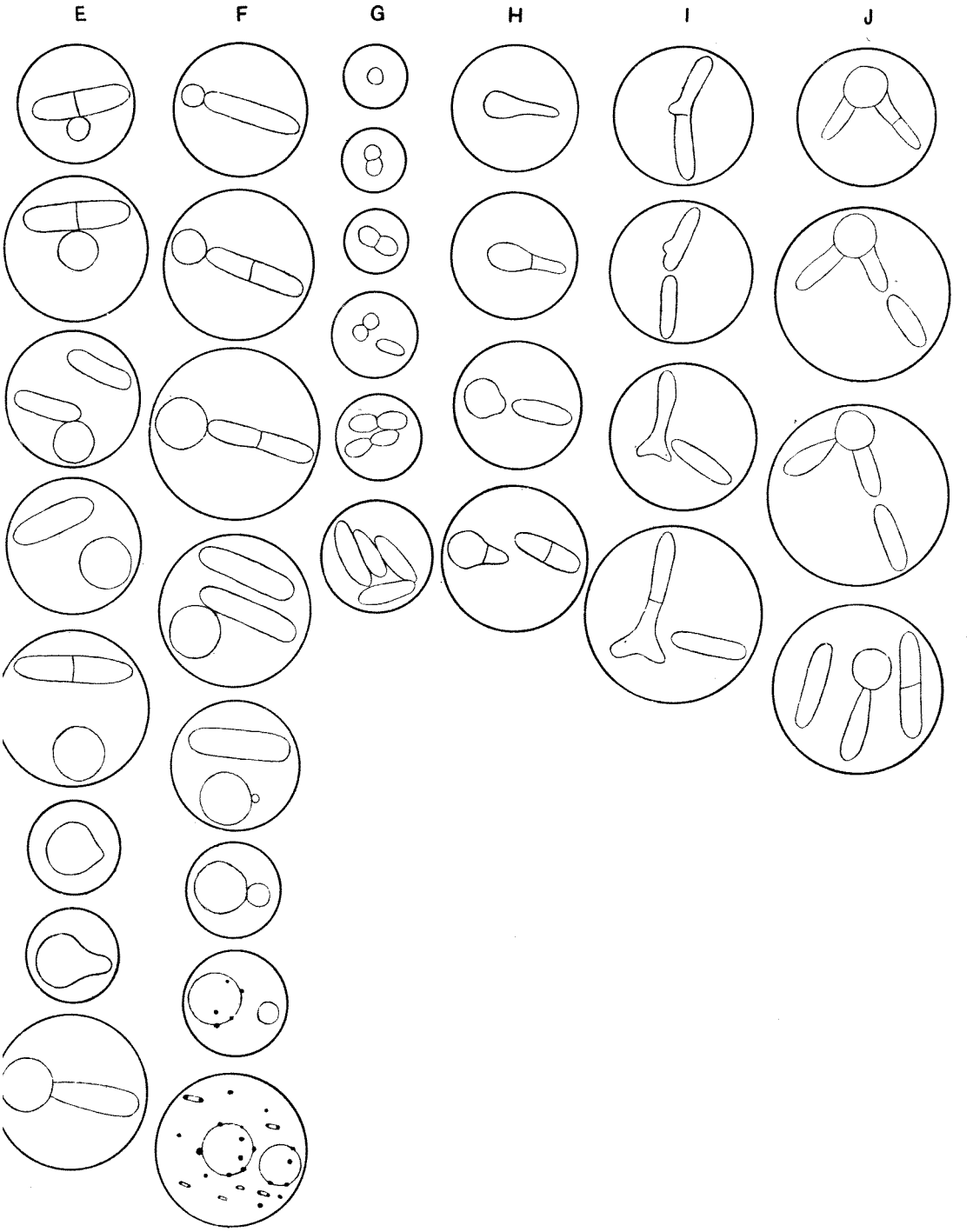

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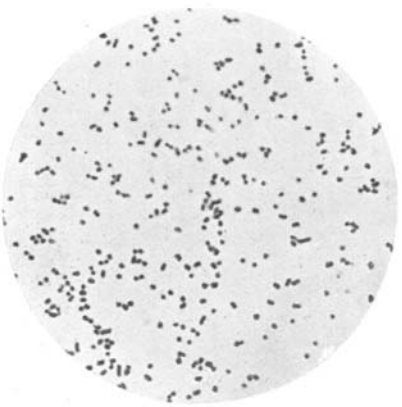

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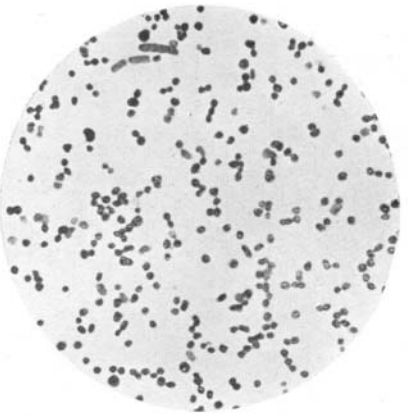

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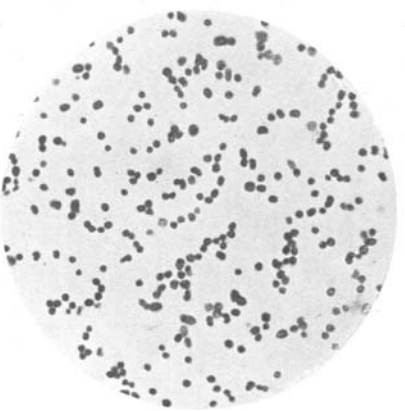

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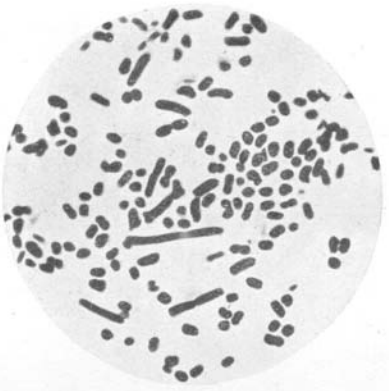
PLATE VII



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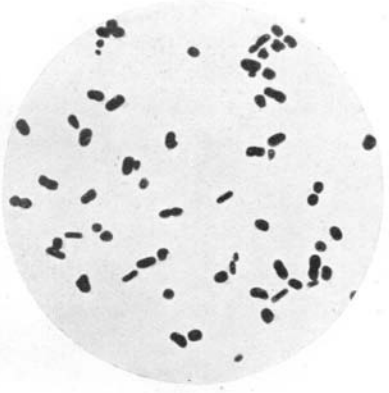

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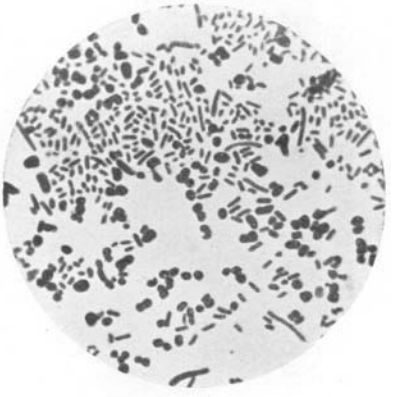

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