A CONTRIBUTION TO THE BACTERIOLOGY OF A FUSO-SPIRILLARY ORGANISM, WITH SPECIAL REFERENCE TO ITS LIFE HISTORY[1]

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This study has as its origin an obscure clinical case of generalized infection with a fuso-spirillary organism. In this communication I do not deem it desirable to allocate its place in the scale of organic life with finality, partly because of present classificatory readjustments that are being carried out by the Society of American Bacteriologists, and partly because of additional work that we are prosecuting with these and related organisms. The term fusospirillary I shall employ generically, although by some it has been temporarily preempted for the fusiform bacillus and the so-called spirochaetes found in Vincent's Angina. This disease and many other related ones will fall within the scope of the series of studies being at present conducted, which it is hoped will prove advantageous to a more fundamental understanding of their bacteriology.

CASE HISTORY AND SOURCE OF THE CULTURE

In the summer of 1916, J. B., sixteen years of age, underwent an operation for appendicitis. The latter condition was found to be complicated with an abscess, which was drained. Convalescence occupied a period of about six weeks. Although the wound healed, the patient did not regain his health, as was manifested by a continuous fever of

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from 100° to 102°. In March, 1917, a futile exploratory abdominal operation was performed, the symptoms directing attention particularly to the right hypochondriac region. The temperature fell to normal for a few days, following the operation, but soon rose to 103°–105°, after which very marked daily variation became the rule. In the morning it was usually normal and at times subnormal, only to be followed by a marked afternoon rise of 103°–105°. At this time pus and albumin were found in the urine, a polymorphic leukocytosis of 17,000 to 20,000 was present, and a bronchial catarrh, which had been noticeable from the first, assumed a more severe character.

Prolonged search of the mucopurulent sputum revealed a few small, opaque, white spherules about 1 millimetre in diameter. They were not at all caseous, but were quite cohesive. They reminded one of the sulphur granules of actinomyces infection. The sputum was rich in lymphocytes. When the spherules were crushed they were found to consist of necrotic masses and a thread-like branching micro-organism apparently in pure culture. These organisms were non-acid fast and Gram-negative. Several months later samples of sputum showed the same cohesive granules to consist of interlacing, very slender filamentous forms which were made up apparently of single rows of tiny granules. They stained poorly in Loeffler's, but very well in fuchsin. Among them were found also some vibrio forms and fusiforms.

On March 28, 1917, a lung puncture was made at the ninth right interspace, in the post-axillary line, at the point of greatest tenderness. A slight amount of serum and flaky material was obtained, resembling the spherules found in the sputum. Examination of the material showed it to consist almost entirely of bacterial growth. There was marked pleomorphism of the elements found. Asymetric bacillary or filamentous forms combined with large circular or ovoid forms predominated. The former varied from 5 to 20 microns long, the latter from 2 to 4 microns in diameter. These circular forms simulate the large giant cocci to be described in the first blood culture. There were also many thin wavy filamentous forms. These organisms grew only anaerobically. In broth the circular forms gradually disappeared leaving filamentous and coccal forms. The latter varied in size from about 0.25 microns to 1 or 2 microns. They usually occurred in groups. Repeated anaerobic plating showed that this culture was probably pure despite the confusing variety of forms.

On April 7, 1917, a second abdominal operation was performed, revealing a renal abscess, the pus from which was aspirated and a piece

of the kidney removed for histologic examination. The pus contained curved bacillary forms, occasionally faintly granular and not infrequently branching. They were frankly Gram-negative and in Loeffler's stained poorly. In Romanowsky they stained deeply blue. There was also present an occasional small group of metachromatically staining, extremely small coccal and diplococcal forms. This pus yielded absolutely no growth on any medium under aerobic conditions. On blood agar, anaerobically after nine days, small elevated colonies appeared. They had precisely the morphology of the Klebs-Loeffler bacillus, but emitted a putrid odor characteristic of *Bacillus fusiformis*. After three days the same pus on hydrocele agar developed greyish elevated colonies of a granular bacillus with pointed ends, which also developed this putrid odor (fig. 1). They had the staining reactions of *B. fusiformis*.

These organisms differ somewhat from the fusiform bacilli usually seen in exudates. Although many have the chromatin disposed as a single granule or as two granules paracentrally located, others show it disposed in rod shape, resembling vibrios. The latter may be S-shaped or straight. This culture stained in Romanowsky showed a delicate faintly staining substance surrounding the bacteria, in mantle-like fashion. It is in reality a periplast, which probably originates from the centrally located vibrio-like chromatin. Forms simulating these have been grown in association with the typical filamentous form and will be discussed further on page 522 (see fig. 2a).

The architecture of the kidney was almost entirely displaced by granulomatous tissue, characterized by fibrous change, and a diffuse infiltration with a large mononuclear type of cell answering the description of the endothelial leucocyte. In many places the infiltration seemed to be made up almost entirely of these cells. Typical plasma cells and polymorphonuclears were also present, the latter being most conspicuous in the remnants of the uriniferous tubules and glomeruli. The histopathology was suggestive of the actinomycotic lesion, but lacked the characteristic colonies. An examination for tubercle bacilli was negative, but a Gram-Weigert stain, cautiously decolorized, revealed most of the forms found in the smears of pus.

On December 7, 1917, one week before the patient's death, he developed multiple pleural sinuses from both pleural cavities. A sample of pus from each side stained in Romanowsky shows the variety of forms already described. There were large numbers of true fusiform bacilli, the giant coccus forms with peripheral granules, the small oval eccen-

trically granulated forms of various staining intensities, and the very thin wavy pink staining forms, some of which can be seen springing from the end of thicker, blue staining vibrio forms. There are also found the interrupted forms—slender curved bacilli in threads but connected by a non-staining link of appreciable length. All the discharges had the characteristic putrid odor of infections with *B. fusiformis* and the lesions were of a sloughing, gangrenous character, as was instanced by the breaking away of the wound stitches, due entirely to the direct but indolent extension of the process into the healthy tissue that held them. All attempts to obtain an autopsy on this patient were unfortunately futile.

The wide morphologic diversity evidenced by both the tissues and cultures favors the interpretation of a mixed infection, yet a careful analysis of the findings, especially the results of the blood cultures, revealed phenomena very inadequately explained by this hypothesis, and led me to seek a more satisfactory one. In the first place, the anaerobic character of all flora obtained from closed lesions (except the blood) was to say the least unusual, in consideration of the marked pleomorphism of the flora of the individual lesion. Most of the forms found were known to be rather closely related biologically. It is of note that the odor permeating the patient's room following the abdominal operation could be compared best with one thing, namely, a culture of *B. fusiformis*. Likewise the culture of *B. fusiformis* isolated from the renal pus as well as the aerobic coccus forms growing from the blood and from the Berkefeld's filtrates of the blood-serum produced this same odor, but the aerobic cultures lost it after the third generation. The fact that the aerobic coccus and the anaerobic bacillary form possessed this biologic character in common also suggested relationship between them.

The clinical course of the disease was akin to that of tuberculosis, or some mycosis. The intermittency of the clinical symptoms was most noteworthy, the patient having experienced about half a dozen clinical cures before he actually died. For weeks at a time he would have no fever, and would gain greatly in weight and strength. Assuming that the condition was mycotic in nature, it seemed reasonable to consider the various

morphological forms as phases in the life history of one organism. Such an hypothesis was particularly serviceable in the interpretation of a very peculiar, and in my experience novel phenomenon, observed in connection with the blood cultures. In its explanation the mixed infection idea was inadequate, and its investigation forms the starting point for the entire study.

BLOOD CULTURES

Ten cubic centimeters of blood were first grown in 50 cc. of broth in two separate flasks of the same medium (broth 8). After forty-eight hours a small amount of granular sediment appeared, but the supernatant was entirely clear. Examination of one flask at this time showed some very large circular forms (giant cocci, fig. 3) varying from 3 to 4 microns, staining faintly or not at all in Loeffler's and negative to Gram, except some granules at the periphery of the cell.[2] Wright's stain as well as cresyl blue were used to differentiate these from erythrocytes or their products. With Romanowsky they stained quite satisfactorily.

Until the fifth day the growth consisted of a slowly increasing, coarsely granular sediment. At this time, however, reproduction occurred with such amazing rapidity that the culture became diffusely clouded in three hours' time, which event constitutes the novel phenomenon to which I have referred. To my surprise the culture then contained a preponderance of usual-sized diplococcus and coccoid forms. The same findings were

[2] At this juncture 2 cc. of the broth (no. 8) were pipetted off this flask and placed at room temperature for a day and then in the ice-chest. In three days, long branching filaments developed, some taking their origin from the intracellular coccoids, others apparently arising from the peripheral granules of the giant cocci (figs. 4. 5, and 6). This culture has been carried for more than 100 generations under varying conditions, but diplococci such as germinated in the flask on the fifth day have never been separated from it. As will be seen, steps were taken to rule out contamination in this instance, and such response to environment is one of many observations favoring the cyclic nature of the changes seen. The cocci developing from the blood in another batch of broth, although suggesting filaments by the short spicules that arose from some of them, would without other evidence have been misleading.

present in the second flask which had not been exposed to the air by examination of it. A second blood culture taken in another flask of a different batch of broth (no. 16) developed these same diplococci and coccoid forms in twenty-four hours. Reference to the media book showed certain irregularities in the preparation of the first batch of broth. In order to ascertain the possible influence of this factor, a third culture of 10 cc. was taken and equally distributed between flasks of broths 8 and 16 with results which are a repetition of those just related. It seemed certain that the broths were quite different, and certainly possible that this difference found expression in the way just related.

Warm stage studies, the results of which will be described presently, suggested that the organism was present in the blood serum in filtrable form. Accordingly 5 cc. of freshly drawn citrated serum was diluted with 25 cc. of sterile NaCl solution, and passed through a Berkefeld N. filter. The filtrate was mixed with the no. 16 broth with the consequent development of the same diplococcus and coccoid forms found before. The luxuriantly growing diplococci were found pure on plating. Further evidence for the existence of the organism in the blood in filtrable form is seen in the fact that a suspension of these diplococci, grown as such, refused to pass the same Berkefeld filter that had previously passed the filtrable stage of the organism through its pores. Furthermore, the identical filter, after being cleaned and sterilized, once more passed the original sample of diluted blood serum and gave rise to the same cocci. The experiment was repeated in order to be sure that the refusal of the cultured diplococci to pass the filter was in no way dependent on a mechanical plugging of its pores during the first experiment. These experiments would seem to show that there existed in the blood serum filtrable forms which germinated into diplococci and coccoids, which forms themselves were not filtrable. But I wish to emphasize the fact that the experiments do not prove that these diplococci and coccoids may not give rise to another order of filtrable body, conditions for whose germination have not been fulfilled. The evidence for the exist-

ence of different orders of gonidia will be discussed later. The filtrable bodies I shall hereafter speak of as gonidia. Entirely in line with their presence in the blood is their microscopic appearance in the pus of the renal abscess. Stained in Romanowsky, they appear as groups of very small pyriform structures metachromatically staining. They are also found, together with the coccus and coccoid forms, in the pus as well as in the tissue of the renal granuloma itself.

A more detailed study of the provisionally designated giant cocci, already referred to as occurring in the blood culture, shows that some of them contain from four to six distinct oval intracellular bodies, similar in size and morphology with extracellular forms of the same size (fig. 5). These latter constitute part of the coccoid forms and are to be distinguished from the typical diplococci and the gonidia. In addition to the oval intracellular bodies, very many of the giant cocci average four chromatin granules, located usually at the poles of the cell.

This latter form has occurred frequently in generations far removed in number from the original blood culture. With Romanowsky the granules stained a deep blue, and were usually clearly defined, while the remainder of the cell was either achromic or varied from light pink in the younger forms to a magenta or frank blue in the older ones (figs. 4 and 7). This staining intensity increased first in the periphery of the cell and was often an indication that the filamentous phase of the organism had been reached. In fact, it can be shown that this peripheral staining intensity often appears to represent the development of intercommunicating filaments between the granules, which later may encroach on the central pink portion until ultimately the whole cell is intensely blue (fig. 4b). Under certain conditions the peripheral granules may develop lateral projections, resulting in a stellate form (fig. 4a). The cell body appears to disintegrate later, leaving the pleomorphic, deeply stained granules attached to long wavy filaments. These later may break up into vibrio and then small coccus forms. Under appropriate conditions, these coccus forms seem to fuse into an amorphous, granular mass, and giant cocci, having the character of many of those

found in the blood cultures will again appear, and the cycle start anew. In fact, the observations on the living forms, as detailed below, were seen in a transplant from such a culture.

WARM STAGE OBSERVATIONS

This method of study was adopted as constituting one of the most direct avenues for the solution of the problem created by the fulminating rapidity which characterized the advent of diplococcus forms into the broth culture. Figures 8 to 11 inclusive show that the giant cocci develop quite definite intracellular changes, the end result of which is the formation and liberation of gonidia. Inasmuch as this entire change occurred in less than half an hour, it might serve to explain the phenomenon above referred to. It seems probable, not only from direct observation but also from the study of stained specimens, that the oval intracellular forms, having on their central end the suggestion of a filament, gave rise to the extracellular forms by a process resembling schizogony among the protozoa.

It is important for a study of these forms that cultivation be carried out in serum or hydrocele broth, as the forms develop slightly if at all on solid media. My preparations were made by pipetting off some of the sediment to a perfectly clean slide, and covering with a cover glass, scrupulously clean, in such manner that no bubbles of air were enclosed, and that no excess of liquid projected from the edges of the cover slip. An airtight cell can then be made by rimming the edges with paraffin, and the preparation usually lasts at least twenty-four hours, if properly made. This is an old and well known method and for this purpose is much superior to the hanging drop; unless the preparation is free from air bubbles, however, much difficulty will be experienced in the necessary stability of the object to be studied. It should not be imagined that these changes can be seen on any occasion or under any conditions. One may search many hours, even days, in properly prepared specimens containing many large forms, before observing clean-cut and definite changes. And when they do occur, they may be of an abortive nature, as indeed happened twice in the specimen described, before the actual cycle depicted was finally consummated.

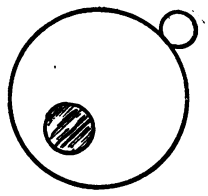

FIG. 8A

FIG. 8B

FIG. 8A. GIANT COCCUS MEASURING FROM 3½ TO 4μ AS SEEN AT 9.00 A. M.

The highly refractile, finely granular central body changes location rapidly, and is actively amoeboid.

FIG. 8B. REPRESENTS THE IRREGULAR, STRUCTURELESS FORM OR RESTING STAGE, FOLLOWING 2 STAGES OF CYCLIC ACTIVITY COVERING PERIOD FROM 9.00 A.M. UNTIL 5.00 P.M.

Resting stage lasted until 10.45 I .M.

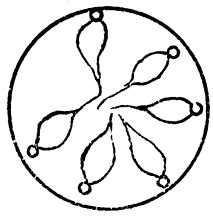

FIG. 9A

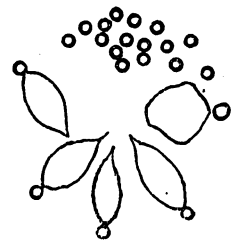

FIG. 9B

FIG. 9A. 10.45 P.M. CELL BECOMES CIRCULAR AND DEVELOPS A DISTINCT WALL

Six oval, highly refractile bodies appear, having a wavy filament on one end and a minute, highly refractile granule on the other. The latter look toward the periphery, the former toward the center. Simulates a protozoal rosette.

FIG. 9B. 11.00 P.M. CELL INCREASES IN SIZE AND WALL BECOMES INDISTINCT

Apparent extrusion of peripherally located granules. Apparent incomplete fusion of 3 oval bodies with resultant gonidial formation.

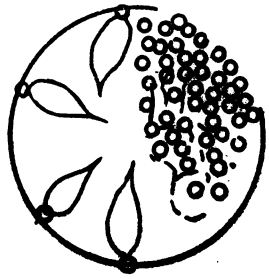

FIG. 10A

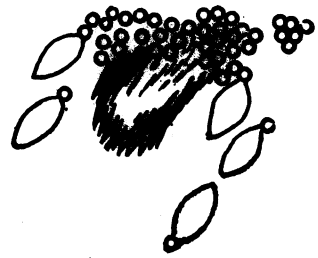

FIG. 10B

FIG. 10A. 11.10 P.M. CELL FURTHER INCREASES IN SIZE

Wall becomes more indistinct, and cannot be made out in the neighborhood of the granules which have increased in number and are actively motile.

FIG. 10B. 11.15 P.M. RUPTURE OF CELL WALL WITH ESCAPE OF GONIDIA

A definite rent appears to three-fourths of depth of cell. Gonidia have rapid oscillatory motion which imparts a movement to the entire cell. Its amplitude of vibration is increased as they are freed into the surrounding medium.

FIG. 11A

FIG. 11B

FIG. 11A. 11.20 P.M. CELL MUCH DIMINISHED IN SIZE, AND OVAL BODIES ARE INDISTINCT

FIG. 11B. 11.30 P.M. THE CELL WALL IS NOW INTACT; CELL IS IRREGULAR IN SHAPE

The oval bodies are imperfectly made out, and little differentiation is present. Cell slowly increases in size until 11.45. It again goes into a resting stage.

Closely related to these forms is another large circular form, usually unstainable, and consequently studied to best advantage in the fresh state. Such forms have been observed under the following conditions: The Berkefeld filtrate of the patient's blood serum developed coccus forms on blood agar, growing readily under aerobic conditions. This culture, after several months' residence in the ice box refused to grow on solid media at 37°. Examination of it microscopically showed what resembled a metachromatic mass of granular debris. When planted at room temperature in plain broth the typical filamentous form of the organism developed, but if planted in broth containing a little hydrocele fluid at 37°, there appeared large coccus forms, which under the warm stage developed cyclic changes similar to the ones just shown. When a drop of this sediment was examined directly in an air tight cell, most remarkable forms were seen. The predominant form is hyaline-like, varies in size from ultramicroscopic to 1 or 5 microns in diameter, and has a clean cut circular appearance. I have observed this form in many cultures under a variety of conditions, and its appearance seems to occur most commonly after apparent fusion and disintegration of the bacterial cells. They are indistinguishable from those adequately described by Hort (1917) in his studies on the meningococcus, although he regards them as ascospores on account of the endosporulation and gemmation and even segmentation which he has observed in them under the warm stage. They certainly give the appearance of reproduction by budding as well as by the schizogony-like process suggested by figure 5. So far, I have not satisfied myself conclusively that gemmation takes place, but I believe it is probable. As Hort has pointed out it is absolutely essential that one study these forms in broth cultures and with wet preparations. To attempt to gain an adequate idea of them with ordinary stains and the use of solid media is to be assured of failure.

I should like to speak of one other morphologic change which is of singular importance regarding the conception of the bacteriology of Vincent's angina and related affections. The symbiosis of *B. fusiformis* and a spirochaete has had traditional

etiologic acceptance. Since 1900, however, there have been a few dissenters, most conspicuous among whom have been Tunnicliff (1905). She contends that the spirilla and *B. fusiformis* are different phases of the same thing. Her principal evidence for the contention lies simply in the fact that in the cultures of *B. fusiformis* certain spiral forms appeared which she feels are identical with the spirochaete of Vincent's angina; but, from her photomicrographs of them as well as from the text, I am inclined to class them among the filamentous and spirillar forms which I have just described. Their width compares favorably with other spirilla and they stain intensely in Loeffler's methylene blue and other simple stains. Such characteristics do not belong to most spirochaetes, including those forms mistaken for such in the ulceromembraneous form of Vincent's angina. These latter stain pink or bluish pink in Romanowsky while both the fusiform and vibrio and spirillar forms of this condition stain a frank blue. We have been able to develop repeatedly in culture the true spirochaete-like forms having the specific staining reaction of the real spirochaete (fig. 12). It goes without saying that they are not genuine spirochaetes; at least I have never observed motility in them. These arise most commonly as lateral or terminal outgrowths from the branching or filamentous form, although more rarely they may spring from the fusiform bacillus itself or they may, under certain conditions, come directly from peripheral chromatin granules of the giant coccus forms. I am not aware that these very slender, wavy forms with the specific staining reaction have ever before been reproduced culturally, from organisms of this class.

Although true spirochaetes may be cultivated from Vincent's angina, as shown by Krumwiede (1913) and others, I do not feel that there is sufficient evidence for regarding them as representative of the majority of the wavy forms found in this condition. Furthermore, by no means all of these forms are motile. There are cultural reasons, as Noguchi (1917) has suggested, for regarding these wavy forms as closely related to the spirilla.

In some experimental subcutaneous and intramuscular ulcers I have observed large numbers of these slender pink-staining

forms, in no way morphologically distinguishable from those of Vincent's angina. They could be seen at times arising from the end of the bacillary form (either diphtheroid or fusiform), and were most numerous when the lesion was nearly healed. It is my contention then, that there are found in these clinical conditions four principal forms: (1) *B. fusiformis;* (2) coarse, sometimes branching, loosely wavy long spiral forms, staining readily; (3) true motile spirochaetes and (4) forms strikingly like spirochaetes in morphology and staining reactions but non-motile, yet easily distinguished from the coarse wavy forms from which they may at times arise, as has been shown in culture. In part from this cultural and experimental evidence, and partly from the fact that in the lesions of the case under discussion spirochaetes were not demonstrated, although fusiform bacilli were cultured from the gangrenous, putrid smelling lesions, and in part for reasons yet to be presented in the study of these organisms, I do not feel that we have adequate evidence for placing a higher etiologic value on the true spirochaetes associated with the fusiforms than we do on the hemolytic streptococci, which may also be associated with them.

GONIDIAL FORMATION

I wish now to present, in brief, evidence tending to show that this strain produces at least three distinct orders of filtrable bodies, and that each form is chiefly, although not wholly derived from a distinct morphologic phase in the cyclic development of the organism. We are in possession of considerable evidence that the granular diphtheroid-like phase develops within itself minute filtrable gonidia, conclusive data regarding the fate of which must be left for future study. The presence of occasional refringent areas located between the staining granules of *B. fusiformis* has been noted by various observers. To a lesser extent these areas are present in diphtheroid bacilli. In the diphtheroid phase of this organism, as well as in other known diphtheroid bacilli, I have observed, under certain conditions, a very marked increase of highly refringent minute circular

bodies, developing in the lighter spaces between the stained bars of the organism. With respect to *B. fusiformis*, it has been stated that the areas are not circular; but, during periods which I wish tentatively to style as gonidio-potent, these spaces seem to be the anlage for the formation of definitely circular bodies.

It is of note that, coincident with a marked intracellular increase of such bodies, very large numbers of similar structures are found free of the bacilli (fig. 13). When growing aerobically, they usually occur in young cultures (six to eight hours) in largest numbers. They appear to stain better as they age, the dye being absorbed first at the poles and on the periphery of the body. They may appear in pairs when free. They may be seen at times on the ends of the organism, and not infrequently bulge from the side, clostridial-like. These considerations suggest strongly the intracellular origin of the extracellular bodies.

Subcutaneous injection of the filtrates into animals has shown results under certain conditions, and more extensive work along this and cultural lines must be done before the nature of these bodies is finally determined. Attempts to grow them have apparently developed forms which appear as very small diplococcoid bodies (stained with Romansky) as well as forms resembling the stellate and giant coccus forms already described. Fig. 14 represents one of the forms, and simulates closely those seen under the warm stage. It is unfortunate that, owing to interruptions in the work, this particular form died out after being laid aside for some months in the ice chest. From their appearance in young cultures, together with their other characteristics, I am inclined to regard these minute bodies as regenerative units, rather than products of degeneration. It seems possible that this process may occur frequently in these organisms, but its extent is greatly influenced by external conditions.

It is not possible at this time to formulate any set of conditions that will with any degree of certainty bring about these periods of gonidia-potent activity. They have occurred under quite diverse and often fortuitous influences apparently, such as the slow evaporation of NaCl solution from a blood agar slant and following transplantation from the depths of an agar shake culture on to a moist blood agar slant.

The French authors, Besson (1913) and Vincent (Besson, 1913), state that these bodies are not spores, inasmuch as they do not have the staining reaction of spores. Tunnicliff (1911) has described spore formation in fusiform bacilli. A personal communication from her indicates that she has based her contention on staining reactions alone, and adds that the bodies may not be true spores. I have noticed a tendency in these granules to retain the stain after decolorization, which has also been shown to be the case with certain diphtheroid bacilli (Mellon, 1917). Although I have made no studies of the tubercle bacillus with respect to the well known granules of Much, the description of the latter inclines me to regard them as comparable to the forms under consideration. These bodies have reacted negatively to the few physiologic tests I have performed with them.

The second order of gonidia is produced chiefly by the filamentous branching forms. They arise either from the end of a main stem or its branches, or directly from the side of either with almost equal frequency (figs. 15 and 2). They stain much deeper than the rest of the cell, and are not resistant to the ordinary stains, as is the first order. Their number depends somewhat on the length of the filament; but, generally speaking, they are more numerous than those of the first order. When a suitable culture was filtered, a finely flocculent precipitate formed in the broth at 37° and at room temperature, which microscopically contained bizarre shaped, irregularly staining bodies, scattered among what was apparently granular debris. Control tubes of this broth did not precipitate, and the nature of this amorphous material cannot be interpreted at present. There is no reasonable doubt concerning the microbic nature of the forms found, as they stain very distinctly; yet, from the fact that they have not yet been successfully transplanted, even though derived from a rapidly growing filament, it is evident that there is much to be learned about them. A variety of forms are discernible. Some are undoubtedly rod forms with rounded ends which are often slightly granular. Others have a diplococcoid appearance, while still others are bizarre shaped,

reminding one of the club-shaped forms of the diphtheria bacillus. It is of interest that none of these forms resemble in the slightest the branching filaments from which they are presumably derived. Fig. 16. Rosenow and Tunnicliff (1913) described small coccus-like bodies in culture from a case very similar to this one. They believe that these coccus forms came from the dilated end of the filaments. They were not able to cultivate them separately, but say they resemble the spores described previously by Tunnicliff. They offer no further suggestions regarding their nature.

The third order of filtrable body I have already described on page 510. They were obtained from the blood serum and also in the third generation of the first blood culture. From the fact that I have not been able to obtain them from any subsequent culture, it is probable that their development in such an early generation is explained by their being carried directly from one culture to another.

Although these orders of gonidia as outlined have developed in connection with these various phases, it is by no means certain that they are always formed in the same way or that bodies answering their description are always seen with the forms described. For example, I have observed occasionally small oval staining bodies in the filamentous forms, and easily staining circular forms on the ends and sides of the granular bacilli. With little regard for size or shape, all spheroidal bodies found in connection with these filamentous forms have received the designation of coccoids. From their difference in behavior it seems probable that there may be a number of bodies of different nature included under this term; and, although it is difficult to speak with assurance regarding all of them, there is a reasonable degree of evidence against all of them being involution or degeneration forms.

CULTURAL AND MORPHOLOGICAL CHARACTERS OF THE DIFFERENT FORMS

The filamentous form occurs with or without branches, varies from 0.5 to 1.5 microns in width, and, in length, from short forms up to those covering one or more fields of the microscope. Under certain conditions large oval knobs may develop at the end of the branches. In common with the filaments, these bodies stain readily and are Gram-negative, and not infrequently contain one or more highly refringent minute bodies, which at times bulge out from their margins. They may occur independently of the filament in which case however, there is usually a short spicule or outgrowth from one or both ends. Optimum growth occurs at room temperature, proceeds slowly in the ice-chest, and may or may not take place in the incubator. The latter temperature seems to conduce to the formation of the terminal oval bodies above mentioned (fig. 6). Beef-heart media seems to be another of the probably large number of factors contributing to the development of these forms.

On slants, growth is luxuriant, the colonies being usually discrete, and after twenty-four hours developing a lemon-yellow pigment on most media. The colonies are raised, moderately moist, fairly adherent, often to the extent of giving the colony a countersunk appearance. In the older colonies, especially, a concentric striation, suggestive of actinomyces, develops. On old, slowly developing slants, coalescence of the colonies is common, and the filaments often fragment into shorter bacillary forms and into diplococci. In transplants the growth is usually filamentous, and it can be said with certainty from warm stage observations that the diplococcus and bacillary forms return directly to the filamentous form on transplantation. In moderately old cultures some of these filaments may lose their staining power and become stippled with fine granules. Similar granules may be seen free of the filaments, and should not be mistaken for gonidial granules, which they resemble closely. Unlike the latter they are not filtrable and are involutionary in nature.

The granular or diphtheroid forms have the characters that are associated with most diphtheroids. They are Gram positive, do not grow perceptibly at room temperature, but in the incubator grow on blood-agar slants, appearing as moist small semi-transparent, discrete non-adherent colonies. In the ice-chest, as a rule, growth is not perceptibly increased; but on some media the whole growth may become changed to large coccoid and diplococcoid forms. In broth, a moderate sediment precipitates from a slightly diffuse supernatant, the quantity of which is increased by the presence of serum. Gelatin is not liquefied. This form is a facultative anaerobe.

The diplococcus form, which may also be arranged like staphylococci, grows luxuriantly on any medium, and its optimum temperature is 37°, although it may grow at room temperature. The growth is luxuriant, white, opaque, moist, confluent and non-adherent on solid media, while in liquid media it grows rapidly and diffusely. Funnel-shaped liquefaction of gelatin stabs takes place along the entire line of the inoculation. It has but slight anaerobic tendencies.

In addition to these forms, all of which grow aerobically, is the anaerobic fusiform type, which is to a slight degree a facultative aerobe. On page 507 I have referred to the periplast formation in connection with these forms. Stripped of their periplast, some of these forms would appear as simple vibrios or filaments. I have encountered, in a culture of the latter, large numbers of what at first appeared to be plump, faintly staining bacillary forms; but more careful study showed many of them to be traversed longitudinally by a filament, or else the latter coincided with the margin of the large bacillary-like form (fig. 2). These forms also occurred at the ends of long filaments, giving the impression that they developed there much in the same way as the larger clubs of actinomyces develop. It will be recalled that the latter have the same general arrangement that I have described for these forms, and I think it fair to regard them tentatively as homologues of the actinomyces clubs. The latter, with the exception of those described by Smith (1918) have never been cultivated, and have been regarded

as a degeneration product of the filament, brought about by reaction of the tissues. In this connection it is of interest that these forms developed in the depths of a culture of "hormone agar," the essential feature in its preparation being the preservation of certain growth products of the tissue employed. The forms did not grow in transplants, and have been encountered inconstantly.

No transition changes have been observed with the fusiform organism found here; hence nothing can be postulated definitely in respect to its relation to the filamentous form. However, Tunnicliff's (loc. cit.) contention regarding the phasic relations of *B. fusiformis* and certain spirilla is suggestive in this connection. Moreover, I have recently encountered true branching in a strain of *B. fusiformis*, which more than ever is coming to be regarded as very closely related to the streptothrices.

INDUCED CHANGES OF PHASE

a. Bacillary to filamentous

It is obvious that, in order to establish the theory that these various forms are phases or stages in the life history of a single organism, it will be necessary to trace the mechanism of transformation of one form into the other. A pure culture of the bacillary form, grown on a blood agar slant, was washed down with sterile broth and distributed over another slant of the same media with a Pasteur pipette. After thirty-six hours at 37°, it was put in the ice-box and, after a month, examination showed a tendency to adhere to the medium. Microscopically, the culture showed coccoid bodies, very variable in size, some being as large as 3 microns. Many diplococcoid forms were present, but only a few of the original granular forms could be seen. Such coccoid changes in diphtheroid cultures are among their best known features.

When these coccoids were sown in broth 8, they developed one or more projecting spicules which, in a few days at room temperature, developed into filaments. Morphologically, no indication of this change was observed among the coccoids of the

blood agar slant at this time. The latter was sealed and returned to the ice-chest for six months longer, and at the end of this time the growth seemed still more adherent than before. Microscopically, many of the large coccoid forms and diplococci showed one or more delicate projections, occasionally branched and of variable length (fig. 17). An occasional bacillary form could also be seen with a long terminal filament projecting from its end (fig. 18). Transplants on blood agar of this culture developed the typical branching filamentous forms. They grew best at a temperature slightly above the room, but also developed in the incubator and at room temperature.

A second culture, having precisely the same origin as the above, after remaining in the ice-chest for the same length of time and on the same media, was transplanted on various kinds of media and under varying conditions, in an effort to regain the bacillary form. All attempts over a course of two months were futile, and it seemed reasonable to assume that the culture was dead; but a transplant on blood agar that had stood at room temperature for a month developed the typical filamentous form. The only varying factor in the treatment of these two transplants from an identical source had to do with the fact that the latter was made in the usual way with a wire, while the former was made from a broth suspension, with a pipette. Still a third transplant made with a wire, the origin of which was in common with the two foregoing, refused to develop filaments after one month's stay in the ice-chest when treated in the same manner as the first transplant, but at a later period developed them when treated in a manner entirely different from the other two. These experiments tend to show the obstructions to any precise formulations of a procedure of this kind, theoretical discussion of which will be undertaken presently.

The mechanism for the development of the filaments seems perfectly clear. The demonstration of their origin directly from the bacillus, as well as from its coccoid forms, and their subsequent cultivation as such, seems to rule out both the contamination and the mixed biotype objections. It is noteworthy that thus far it has not been possible to grow the bacillary form

when the culture had changed sufficiently to make transplantation of the filaments possible. In one instance, the change occurred in ten days, yet the bacilli failed to grow in transplant. In control cultures where no such change had taken place, they grew readily, even after much longer intervals.

As I have already noted, the bacillary form has died out, which fact constitutes the only reason why attempts to confirm these results were not made with a pure line, i.e., a culture started from a single bacillus. Yet it is obvious that if gonidia are actually associated with this form, the purity of the line would still be somewhat in doubt. In the many observations that I have made of the pleomorphism of the branching form, I have never observed the large coccoids developing at low temperatures, such as occurred with this diphtheroid form. On the other hand, the filaments almost invariably developed very small diplococci, as involution forms, both in the ice-chest and at room temperature. It is beyond question that slender wavy filaments do develop from diphtheroid forms at times (Mellon, 1915), as well as from *B. fusiformis* (Tunnicliff), but their significance has been an open question, and cannot be considered settled yet. Of interest also in this connection is the demonstration of filaments in the blood of a patient suffering from a severe fusiform infection (gangrenous balanitis) (Mellon, 1919).

b. Coccus to filament

Reference to the blood culture experiments shows the blood serum of the patient was repeatedly filtered through a Berkefeld, and each time, coccus and coccoid forms were obtained. They varied much in size and stained somewhat irregularly, particularly in Gram, where different degrees of reaction to the stain were apparent. Some of the partially decolorized forms contained deeply blue staining granules. Such features in my experience have always suggested that I was not dealing with a stabilized coccus form, but rather with a phase of a higher organism. This culture was repeatedly plated out at 37°, at which temperature it grew luxuriantly; but before a colony was selected

for transplant, it was placed at room temperature for a few days and then in the ice-chest, so as to give opportunity for the development of any mixed forms. This culture was frequently transplanted for ten months on plain agar. It was then planted in an 8-inch tube of no. 8 broth containing some sterile hydrocele fluid. In twenty-four hours, examination of the diffuse growth showed pure cocci. After three days at room temperature, the growth began slowly to change to a flocculent character, at which time the cocci showed a great variation in size, as well as granular staining with slight projections from one or both ends of numerous organisms. At this time, the culture contained numerous non-staining hyaline bodies, some of which had secondary and tertiary circular bodies attached to them, suggesting a budding process. They were best observed when examined in a wet non-stained preparation. Four days later, there were numerous branched filaments in the culture. The original culture of the cocci on plain agar or blood agar continues to grow as such (twenty-four months).

The culture was then transplanted on a solid medium at room temperature in the hope of developing the filaments. After forty-eight hours, nothing but cocci appeared. The culture was then sealed and placed in the ice-box. At the end of four months it showed no macroscopic change, but a microscopic examination gave the appearance of a fusion or degeneration of the coccus forms, poorly defined granules appearing in a non-staining matrix, with Loeffler's blue. Stained in Romanowsky, a great variety of cocci appeared, both as regards size and staining variations. The matrix now stained metachromatically, and was peppered with fine cocci, many of which showed early developing filaments or sprouts (fig. 19). Transplants of this culture on solid media and in broth at 37° gave no growth in a week but, at a temperature between 25° and 30°, developed in three days the typical adherent chromogenic colonies, showing microscopically long branching filaments. Furthermore, all attempts to plate out the coccus form from the filaments have so far been futile.

Inasmuch as neither the filaments nor their involution forms (fine diplococci) were capable of passing my Berkefeld bougies, the only possible explanation of a mixture in this culture seemed to lie in the gonidial granules derived from the filaments. It is, of course, conceivable that they may have been present in the patient's blood. Filtration of the first, as well as distantly removed generations of these cocci, has never yielded a growth of any description, so it would seem that we can dispense with the possibility of a mixture in this instance. However, it should be noted that, of several batches of broth used in these experiments, broth 8 is the only one with which we have been successful in developing the filaments. It is to be remembered that this was the same batch of broth which made possible filamentous development from the blood culture, when other batches gave rise to cocci only. The perfection of synthetic media will, it is hoped, make possible the formulation of conditions, the inconstancy of which has been a formidable source of confusion in studies of this kind.

Associated with these apparently *direct* morphologic transformations, selected because they leave almost nothing to the imagination, are subsidiary ones whose rôle is probably intermediate in nature. Under a variety of conditions and frequently in young cultures, an apparent fusion of the cellular substance of the forms takes place, accompanied by certain staining changes and the development of other forms whose nature is still undemonstrated. These changes simulate what Löhnis and Smith (1916) speak of as symplastic. Almquist (1917) has described similar changes occurring with *B. diphtheriae* and other organisms. They are under further study at present; and, without making any dogmatic assertion regarding their nature, I am sure that the extent and frequency with which they accompany the more direct transformations presages for them a significant rôle in this process.

DISCUSSION

I wish it to be clearly understood that I do not consider these observations as sufficiently complete in themselves to be conclusive proof of the type of cyclic change that I have hypothe-

cated as a working basis. It is obvious that there are many "missing links" in the chain of evidence. Yet the same comment applies in even greater degree if one attempts to explain the observations as a whole in other ways. I am frank to admit that our present knowledge of bacteriological media is not sufficient to enable one to produce some of the reported findings at will; yet it is pertinent that, while certain batches of the media "held out," it was possible to verify them repeatedly.

It is obvious that there are other explanations for the changes described, the commonest, of course, being that the forms are a mixture of different species. Since Barber's invention of a method for the isolation of single bacterial cells, its application in the concrete case has been accepted by many as prerequisite for the demonstration of biologic transformations. It is unquestionably a valuable method in such studies, but, like other single methods, has its limitations, which are more evident to those who have actually used it, perhaps, than to others.

It should be obvious, even to those who sponsor it with a minmum of discrimination, that its usefulness is, roughly speaking, directly proportionate to the size of the organisms which one desires to isolate; and when their size renders them invisible, or nearly so, its categorical quality disappears. It would appear that, to a certain extent at least, these are the conditions prevailing here; and, although the single-cell method is being used where it seems most applicable, its sphere of usefulness can be more precisely delimited only when we learn more of the nature of the filtrable bodies associated with this organism.

There is good reason to believe that otherwise pure cultures of bacteria are often mixtures of biotypes, and such an explanation is usually sufficient in the minds of some to account for *any* experimental transformations. Changes in environment may bring some one of these types into active growth and suppress others, changing very markedly the morphologic picture of a culture.

It is inevitable that this theory of selection should be invoked to explain many phenomena to which it may bear a remote relation, in the same way that Ehrlich's theory of receptors has,

for so many years, retarded advances in immunology. A fair example of such an interpretation is seen in the recent work of Eberson (1918) on the diphtheroid group. The work (Mellon, 1917) which he refutes, is comparable to the subject matter of this paper; yet, approaching it from one angle only, he aligns it with this facile explanation, without even considering the possibility of a cyclic interpretation. In a future communication, it will be possible to focus a more detailed discussion on this point, in the light of this and allied investigations.

It is perfectly obvious that the various phases of this organism are particularly susceptible to changes in environment, yet it should be remembered that the organism itself is not entirely a passive medium. The extent and nature of its response to environmental conditions are functions of its own inherent qualities, which are handed down from generation to generation. However, it is entirely probable that changes may take place in a race of organisms, entirely in accord with their inherent capacity of response to environment, and those changes be so slight as only to become discernible when attempts are made further to modify them in a given direction, which must, of course, be in accord with their capacities for change. Whether we consider such change to occur in a clone, or whether it be the expression of a mixture of biotypes in a culture, bears on our problem only insofar as to require a different combination of environmental factors to evoke the organism's inherent capacities in a certain direction. An instance will point the meaning: reference to the experiments dealing with the development of the filamentous from the bacillary form shows that one cannot always rely precisely on the same means to bring about this result, even though the source of the culture is the same. Even in an organism that has inherent qualities for progressive change, the environmental limits afforded by the usual artificial medium are often inadequate for their evolution. In this instance, the demonstration of filaments arising from the ends of the granular forms leaves little doubt as to their origin.

Furthermore, when cultures become adapted to such conditions, it becomes a matter of increasing difficulty to induce pro-

gressive changes—they become stabilized, so to speak. I have noticed this particularly in attempting to develop the highly refringent intracellular bodies of the granular form; but the filamentous form is more tractable in this respect.

MUTATION

Ordinarily, changes of the sort typified by the bacillary-filamentous transformations would be viewed as mutations, although the whole subject with respect to bacteria seems to lack unanimity of opinion. If we adopt the more conservative idea that mutants only occur under constant conditions, some other interpretation must be forthcoming for these changes, which are to so great a degree a function of environment. Fermentative differences have often been interpreted as evidence of mutation when occurring among the lower bacteria. The literature contains many instances of such in the colon-typhoid group. Hort (1917) has brought to light much evidence for the occurrence of cyclic change in this group, and would in this way explain the so-called mutations. Since reproduction by equal binary fission has always held sway as practically the sole method of bacterial division, the presence of biotypes in cultures could be best explained by mutation; but, if reproduction can occur in other ways, even in a slight degree, the presence of mixed types or variants may have a more simple and satisfactory explanation. The fuso-spirillary organism has the advantage of being on the borderline between the lower and higher bacteria. It is well known that the latter forms have a complex life history, while the so-called branching involution forms of the diphtheria and tubercle bacilli have always suggested close relation with stabilized branching forms.

INVOLUTION AND PLEOMORPHISM: RELATION TO THE WORK OF OTHERS

Finally, it may be considered that this organism represents an example of pleomorphism. This was the explanation advanced for the transformation of certain diphtheroids to diplo-

cocci (Mellon, 1917). Although more marked than the usual pleomorphic changes, they did not seem to merit the designation of the more pretentious process of mutation. Their cyclic nature was suggestive enough to cause this, a similar study, to be approached with those considerations definitely in mind.

Bayon (Besson, 1913) has described an organism from the lesions of leprosy, that has very pleomorphic characters. He says it assumes one of three forms:

> (1) A non-acid-fast and non-acid-resisting streptothrix; (2) a pleomorphic, acid-resisting diphtheroid bacillus, and (3) a definitely acid-fast bacillus, indistinguishable from the bacillus in the tissues.

Williams (Besson, 1913), also grew an organism from leprous lesions, and describes the following forms:

> A, on broth media and on potato-broth, a non-acid-fast streptothrix in the mycelial stage which produced acid-fast rods; B, on milk and lemco-broth, a non-acid-fast diphtheroid bacillus which also produced acid-fast rods; C, on Rost's medium, an acid-fast bacillus which is but the broken-down stage of a streptothrix which was cultivated from a leper passed through respectively all the stages described above.

Dick and Tunnicliff (1918) describe an organism (*Streptothrix putorii*) isolated from a case of rat-bite fever, which resembles this one in many respects. It is noteworthy that they obtained a diplostreptococcus from the blood in the first cultures, which agglutinated with the patient's serum in 1:80, while the streptothrix itself reacted in but 1:20 dilution. We have no way of knowing definitely whether this coccus may have represented a phase in the life history of the organism; and I desire merely to mention it as a possibility, there being no good reason why streptococci might not be present in such cases as well as the additional ones to which I am referring. Middleton (1910) and Douglas, Colebrook and Fleming (1918) have isolated respectively a diplococcus and a *Streptococcus pyogenes* from similar cases, and in the latter instance the organism agglutinated the patient's serum in a 1:60 dilution.

Smith (1918) has recently described a pleomorphic bacillus from the lungs of calves simulating actinomyces. This organism seems to have certain features in common with the fusospirillary organism, chief among which is the cultivation of several distinct morphologic forms which he interprets, not as due to a mixed culture, but as having a cyclic relationship. Certain progressive changes obtain in his culture, as instanced by the complete disappearance of the bacillary form and its replacement by a coccus form. These bacilli, when associated with the club forms, also disappear simultaneously with the development of changes in the latter. Again, they have in common the quality that certain forms resistant to culture are developed from actively growing forms. The development in my cultures of forms that may be homologues of the clubs of actinomyces is another point of contact in the studies. Those of Smith's organism are, of course, real clubs, while the ones I describe are much smaller, but apparently of the same respective origin. The organism described by Lignieres and Spitz (Smith, 1918) and regarded by Smith as practically identical with his organism, develops bacillary forms, which later give rise to diplococci and streptobacilli.

Park and Williams (1910) show pictures of diptheria bacilli, some of which simulate the stellate forms of figure 4. Their description of them betokens the fact that, under the conditions of the experiment, namely, prolonged growth in broth with occasional mechanical disturbance of the pellicle, changes in the reproductive mechanism occur, which suggest to them the process of autogamy and, furthermore that, when the cultures reach this stage, division proceeds *solely* in these involution forms. The fact that similar "branching involution forms" can actually be made to go further and reproduce themselves as branching filaments, is evidence that their observations may admit of a different interpretation in some instances. It would seem that the change produced in the broth by the growth of the organism sooner or later inhibits or prevents development of the organism in its bacillary form; yet its adaptability is expressed in atypical reproductive changes which result in new forms, the latter being the only forms to divide thereafter.

There is no justification at present for saying that all members of the diphtheria group could be made to produce branching filaments, even though the diphtheroid-like phase of this organism in question differs in no essential particular from diphtheroids as we now know them. Still, it should be emphasized that, even as regards the organism with which I am working, it is essential, before the new forms will reproduce as such, that two conditions be fulfilled. The induced changes, typified in part by the above description of Park and Williams, must proceed past a definite point in the evolution of the organism or, on transplantation, the old forms and not the new ones will multiply; secondly, that transplantation must be made to an environment suitable to the newly developing forms, inasmuch as the change in the original medium presumably conditioned their development.

In essence, both the processes of pleomorphism and involution predicate the return of the altered forms to normal on return to "normal conditions." In light of the two considerations just adduced, it repeatedly becomes a question in my mind whether these pleomorphic forms always returned to normal, or whether there occurred a preponderating development of the original forms, the pleomorphic forms either refusing to develop or reproducing so slightly as to be over-looked. The well known selectivity of various media for certain forms would also speak in favor of such a theory.

Furthermore, the apparently lethal effect of residence of cultures in the ice-chest I believe may often be explained in this way: that they refuse to grow, not because they are dead, but because their protoplasm has undergone changes which refuse to respond to the conditions of growth which are not the counterpart of those that have taken place in the organism. I have verified this theory in quite a number of instances when working with organisms of this group; and, without attempting to formulate a rule in accordance with this and associated factors, I may say that temperature changes have been a most potent agency for the induction of evolutionary changes.

RÉSUMÉ

There has been isolated a fuso-spirillary organism from a case in which it caûsed generalized infection, the point of origin being presumably in the appendix. Its branching filamentous forms relate it closely to the streptothrices, while its bacillary and coccal phases, if we regard them as such, relate it to the lower bacteria. It is noteworthy that the branching filaments were not cultivated from the renal abscess or the lung puncture, although many of them could be demonstrated from the material in both locations—indeed, they constituted the bulk of the flora present in the lung puncture material. They were cultivated from broth blood cultures, however, partly as the result of an irregularity in the preparation of the medium and partly from a radical change in the environmental conditions at a certain stage in the culture's development.

The phenomenon of pleomorphism, undoubtedly well founded, may not explain all morphologic changes in bacteria. Some of those occurring in this strain I am inclined to regard as evolutionary rather than involutionary. As evidence for this view, take for example the demonstration that the filaments originate directly from the body of the granular diphtheroid forms, and can subsequently be cultivated in purity. Furthermore, filtrable forms have been demonstrated in the blood of the patient and in culture, although the ultimate fate of but one order of them has been shown. Warm stage observations have yielded valuable information regarding the origin of some of the filtrable forms, and thrown light on the cyclic activities of the organism. This method is much superior to the single-cell isolation method in a study of this kind, mainly because of the relative invisibility of some of the forms.

I regard the process of phase changes, so to speak, as a function of the environment; but the quality which makes such response possible belongs intrinsically to the organism. Tentatively, at least, I cannot regard these as mutation changes. The latter have been adduced to explain the origin of biotypes from pure lines of bacteria. If one regards mutation conservatively, as of rare occurrence, cyclic changes may offer a better explanation for their origin. Viewed in this light, perhaps some of the

hopeless numbers of bacteria which have received designation as separate species may ultimately come to be considered in some more collective way.

No method can, at present, be accurately formulated for the induction of such changes, not only because there is as yet no such thing as a standard medium, strictly speaking, but even more because of the fact that the stability of bacterial protoplasm is, after all, not absolute. Such lability as it possesses is, however, an intrinsic quality and is not a formidable barrier to the well known quality of cultures to breed true to type—that is, to laboratory type. The transitions as outlined have been repeated many times, but almost never has it been possible to repeat the process in precisely the same way.

Grateful acknowledgment is made to the Directors of this Institution, whose far-sighted policy is responsible for the erection and equipment of an adequate Department of Laboratories, making possible work of an investigative character. I wish also to acknowledge the loan, by the Bausch and Lomb Company, of an apochromatic 2-mm. objective for photographic work, in lieu of one previously purchased but not deliverable until after the war. I wish also to acknowledge the helpful coöperation, clinically, of Dr. S. W. Bradstreet, the physician in charge of the patient on whom the studies were made.

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EXPLANATION OF PHOTOMICROGRAPHS

FIG. 1. Fusiform bacilli grown anaerobically and in pure culture from the renal pus. At *a*, the chromatin has a rod-shaped distribution with a delicate protoplasmic prolongation at either end. Stained in Romanowsky. × 800.

FIG. 2. At *a*, is a form which may represent an homologue of the actinomyces club. Beginning at the left, the deeply stained filament takes a somewhat diagonal course through the periplast, but at the right is marginally located. The faintly staining periplast is divided into three sausage-like loops, the latter of which extends beyond the end of the filament. The remaining two filaments in the field show latterly budding gonidia of varying size. Some larger coccoids are free. Carbol-fuchsin. × 1600.

FIG. 3. The smaller type of giant cocci developing in no. 8 broth blood culture after forty-eight hours. Romanowsky. × 800.

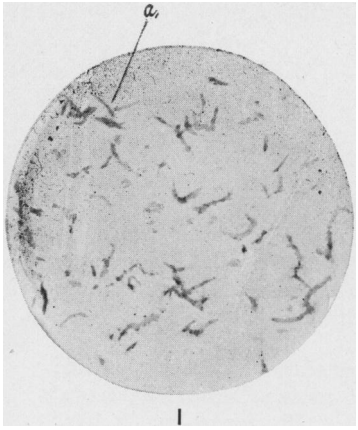
FIG. 4. At *a*, giant coccus forms with peripheral granules one or more of which later give rise to filaments; at *b*, the filaments are further developed. Other smaller coccoid forms of varying size can be seen. These forms have been developed in culture, but represent another type of giant coccus found in No. 8 broth blood culture. Romanowsky stain. × 800.

FIG. 5. In the center of the field is a still larger typ eof giant coccus found in no. 8 broth, blood culture. Four oval intracellular bodies with an early spicule or filament arising from one of them; it crosses the clear sector of the cell at the lower part of the figure. Figure simulates a protozoal schizont. Romanowsky. × 800.

FIG. 6. Filamentous form showing true branching. At *a*, is seen a group of oval spore-like bodies, simulating protozoal flagellates. Romanowsky. × 800.

FIG. 7. In the upper portion is a group of bodies averaging from four to six, peripheral granules. At *a*, early formation of inter-communicating bands between the granules. Many of the latter are free. The lower portion of the figure shows a giant coccus with two small granules on the periphery. Romanowsky. × 1600.

FIG. 8 to 11. Inserted and described in text.



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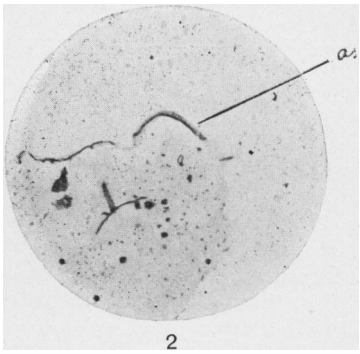

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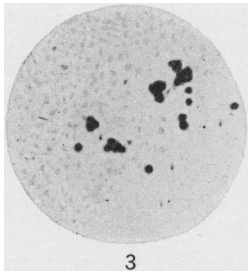

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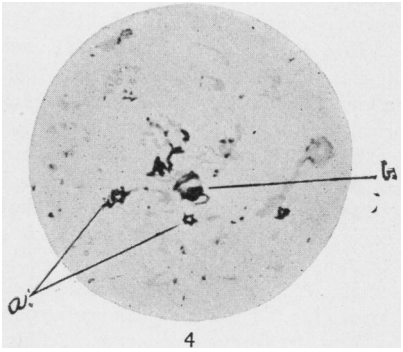

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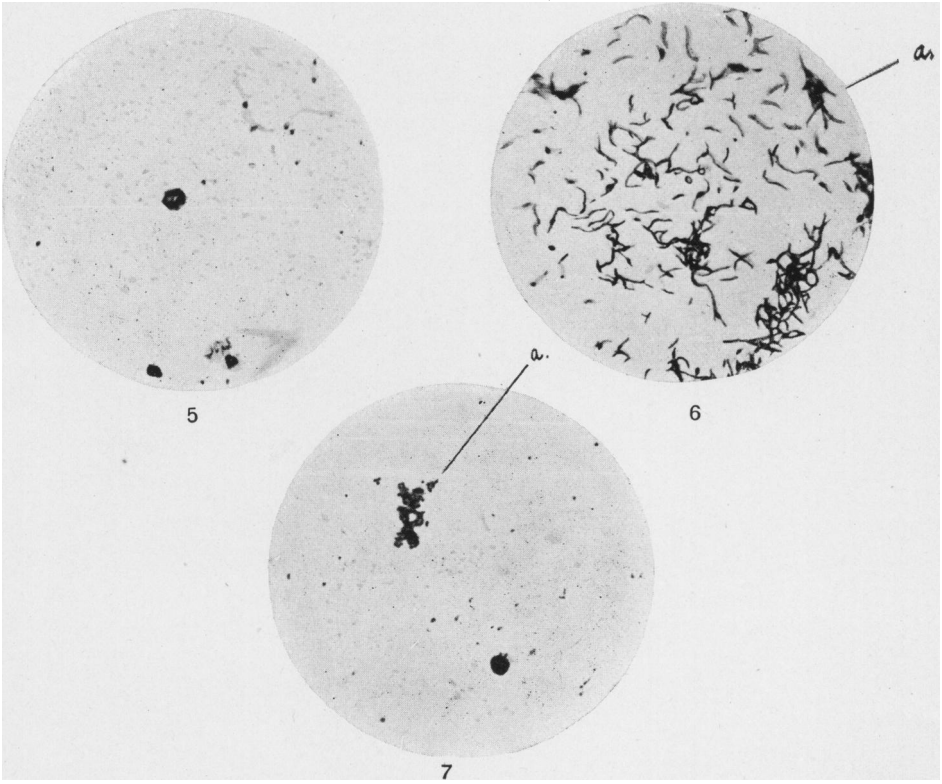

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FIG. 12. Spirochaete-like forms in culture. At *a*, they apparently arise from the bacillary form. Romanowsky. × 800.

FIG. 13. Granular diphtheroid form developing gonidia, many of which are free. At *a*, one is undergoing lateral extrusion. From a twelve-hour blood-agar slant. Specimen overstained in Carbol-fuchsin to bring out the extracellular forms. For this reason little differentiation is seen in the bacillary forms. Carbolfuchsin. × 800.

FIG. 14. The large pear-shaped body in the center of the field developed in a Berkefeld filtrate of the first order of gonidia. It contains several light oval intracellular bodies and two large chromatin granules. Romanowsky. × 1600.

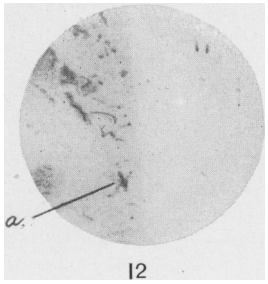
FIG. 15. At *a*, is a filament showing three budding gonidial granules: one is terminal, the other two lateral. This culture was rich in extracellular gonidia, a few of which can be seen in this field. The result of a Berkefeld filtrate of this culture is seen in the next figure. Carbol-fuchsin. × 800.

FIG. 16. The more intensely staining pleomorphic forms represent the results of germination of the gonidia shown in figure 15. Carbol-fuchsin. × 800.

FIG. 17. Large coccoid forms derived from the diphtheroid phase showing early development of filaments; at *a*, one develops from a granular bacillus. Carbol-fuchsin. × 800.

FIG. 18. Shows the origin of the filamentous form from a typical diphtheroid form at *a*. Diplococcoids are also present. Carbol-fuchsin. × 2500.

FIG. 19. Early formation of filaments from the diplococcoid phase. Note difference in average size from the coccoids of figure 17. Carbol-fuchsin. × 800.



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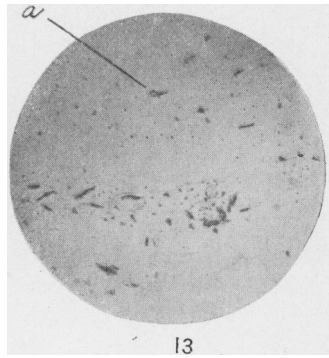

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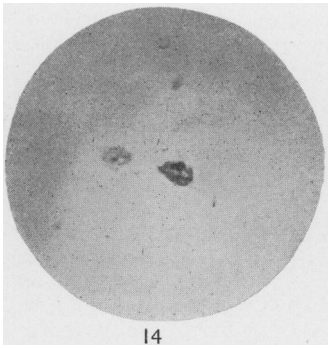

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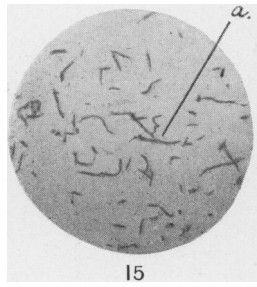

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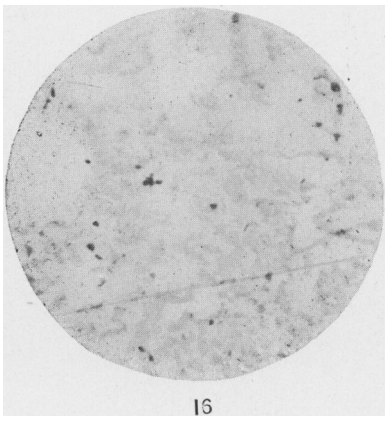

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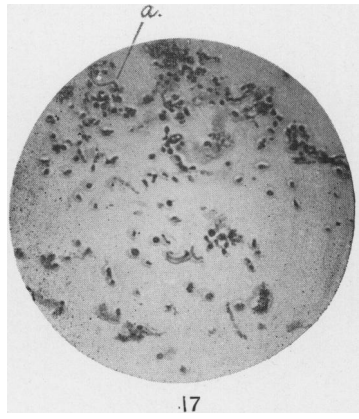

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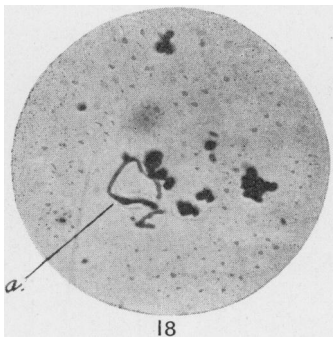

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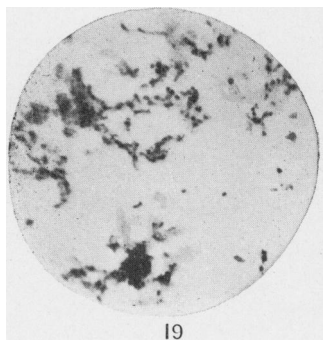

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