ON THE CLASSIFICATION OF THE STREPTOTHRICES, PARTICULARLY IN THEIR RELATION TO BACTERIA.*

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Plates 8 to 10.

The large group of fission fungi, or Schizomycetes, has been for the last half century the center of extensive study in its general relation to disease. On the other hand, the moulds or filamentous fungi, the Hyphomycetes, have received less attention and are, in general, less well known as to their structure, relations, pathogenic power, and occurrence.

Among the conditions making a study of this group difficult are the diverse opinions and practices as to nomenclature of both species and genera. This causes great confusion in the classification of the infecting organisms, and, added to a lack of a uniform standard of characters, leaves the group in a somewhat chaotic state. Several generic names have been and still are used by different authors,—Actinomyces, Discomyces, Nocardia, Streptothrix, Oöspora. Although botanically the matter is not yet settled and further knowledge may make a change necessary, it seems best to follow those who adopt the name Streptothrix for the genus and Streptothricosis for the disease produced. A full discussion of the facts and authorities supporting this view are to be found in the works of Foulerton (1) and Musgrave, Clegg, and Polk (2). The latter authors give the following generic characters for Streptothrices: "Branching filamentous organisms, which develop into colonies made up of organisms and their transformation products. Terminal hyphæ may or may not be radial and may or may not have clubs. The group in general is gram positive and many are

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acid-fast." In a recent article Foulerton (3) describes the type form of this genus thus: "A tangled mass of branched mycelium, as found in pus and other discharges. The mycelial stage is followed by segmentation and fragmentation, producing bacillary forms, and, in artificial media, by chain sporulation; a process similar to this is also probable in isolated rod segments. Old cultures show bacilli, long, short, spiral, wavy, diphtheroid, and paired or isolated spores. All are strongly gram positive and look like bacilli and cocci. They may be acid-fast or not. Rod segments produce mycelium in cultures and tissues. They may produce rod segments direct in animals, but this has not been demonstrated."

Difficulties are likewise met in the identification of species. There have been no well established criteria for them. The limits of species variation are neither set nor well known. The descriptions of many species in the literature are incomplete and often not comparable, since sometimes morphological, sometimes biological qualities, or again each in part, have been used as the basis of species determination. Recent studies by Musgrave, Clegg, and Polk (2), and Foulerton (4) show some pathogenic species to be well defined, *i. e., Streptothrix eppingeri, Streptothrix maduræ, Streptothrix bovis, Streptothrix capræ, Streptothrix nocardii, Streptothrix hominis.* There are also a number of supposedly saprophytic, non-pathogenic forms, isolated from both air and water and other sources. However, the limitation and variation in the pathogenic and parasitic power of these organisms are not well known and animal experimentation with them is admitted to give inconstant results. Pathogenicity, however, is not a reliable specific character, infection being a process always as dependent on the resistance of the host as on the virulence of the organism.

The cause of this confusion and for these diverse opinions and practices lies in the extreme morphological and biological variability of these fungi. Some strains grow freely on all media, some only on special kinds, some apparently cannot be cultivated. This is especially true in isolations from cases of infection, many failures being reported in these attempts; yet when once established on laboratory media, these same reluctant strains may grow freely. This makes a classification from biological characters very difficult.

Much variation is to be found in the morphology of any given organism. It changes its appearance with differing culture media. The liquid and solid state, dryness and moisture, influence its growth. The age of the individual culture and the time since isolation from the body must also be taken into account. The well known granules or "Drüsen," a branched mycelial mass, fragmentations into apparent bacilli and cocci, true spores as well as the minute structures left after chain sporulation, may all be found in the life history of one species. While all the mycelial products are gram positive, the old degenerated threads may fail to stain and only the granules within show the typical blue black color. The exact conditions giving rise to the acid-fastness belonging to some of these species both in test-tube and body are also unknown, and it may be regarded as the development of a more resistant form acquired by only certain products of the mycelium in some species, and by almost, if not quite all in other species.

The extensive literature on this subject contains an immense number of case reports and accurate descriptions of the pathological findings in the body, also many purely biological studies of the organisms and their properties, some species determinations, and more or less complete classifications. A clear history of the subject, since Bollinger's discovery of the fungus in 1877, is given by several writers, Ruhräh (5), Hektoen (6), Hodenpyl (7), von Baracz (8), Musser (9), Foulerton (10), Musgrave, Clegg, and Polk (2), and others. The last named authorities have a bibliography of over fifteen hundred articles; several other authors also give long lists of references.

Among the many clinical and pathological problems that come before the reader of this literature is the biological one as to the possible extension of the genus to include the so called acid-fast group that contains the organisms of leprosy (presumably), tuberculosis, and other well known forms. Sanfelice (11, 12) maintained their structural similarities and divided the genus into three groups, one non-acid-fast, a second partly acid-fast, and a third absolutely acid-fast. Recently Much (13) and Wills (14) have been working with the acid-fast group and by serum reactions have shown the close relationship of its members. Foulerton (3, 4) is a

warm advocate of the generic identity of the organisms of tuberculosis and leprosy with the streptothrices, and his extensive and valuable contributions to the subject entitle his views to careful consideration.

A few workers have applied the recent serological tests to this group with varied results. Widal and his collaborators (15) carried out the diagnostic tests of agglutination and fixation with sera from actinomycotic and sporothricotic cases with success. The spores of *Sporothrix beurmani* were used for the agglutination experiments, and 163 presumably non-fungoid cases as well as cases which were known to be caused by fungoid infection were examined critically for serum reactions. The results were quite definite in showing relations between the fungoid infections.

Choukévitch (16) used the agglutination test with rabbits immunized during several months to show the races of actinomyces; he also produced precipitins, and found specific group reactions in all cases. Harbitz and Grondahl (17), on the other hand, report entirely negative results, but their methods were in all probability defective.

The present work grew out of the study of a series of clinical cases of lung infection that came under my observation. The confused condition of the group made it difficult to obtain a good basis for work, and it appeared possible that some serological experiments might help to clear up matters.

As preparatory to a classification by immunological methods, I examined critically a number of different species of streptothrices, both morphologically and culturally. The strains used were obtained from the Pasteur Institute, Paris, the American Museum of Natural History, New York, and Dr. Foulerton, London. One strain I isolated from a case of lung infection now under observation. The following are the organisms represented: *Streptothrix eppingeri, Streptothrix nocardii, Streptothrix caprœ, Streptothrix gabritschewsky* (Pasteur Institute); *Streptothrix bovis, Streptothrix maduræ, Streptothrix pulmonalis, Streptothrix asteroides* (American Museum of Natural History); No. 585, No. 1276 (Foulerton); and No. 1 (E. C.). The last three strains probably represent types of *Streptothrix hominis III* and *IV* (Foulerton), but

vary somewhat. To these were added *Bacillus leprœ,* the chromogenic form of Clegg and of Duval (18), and the human strain of *Bacillus tuberculosis.*

All workers have encountered difficulty in isolating one of these organisms from a body discharge, especially from sputum, where the secondary invaders almost invariably crowd out the slowly growing streptothrix. This difficulty in isolation is so marked that in relatively few of the clinical cases have cultural studies been made.

After many failures, I succeeded in isolating a pure strain from my case (No. 1) in the following way: About two dozen of the small granules were picked out of the sputum with a needle and washed thoroughly in a sterile tube with sterile water, replacing the liquid several times. They were then injected subcutaneously into a guinea pig. The injection was repeated, if necessary, until a small abscess resulted, which, after eight or nine days, was carefully opened and the pus planted upon tubes of Bordet's potato blood agar. A pure culture of the streptothrix was readily isolated, as the secondary organisms were found to have been killed, leaving the slowly growing one. While at first slow to grow and sporing very early (two to three days), the strain gradually acquired saprophytic power and now grows freely on all ordinary media.

The various organisms mentioned were cultivated on Bordet's potato blood agar medium, on Dorset's egg medium, and on plain and glycerin agar. Preparations from both old and young cultures were stained by the gram stain and for acid-fastness by carbol fuchsin, decolorizing with 25 per cent. nitric acid (table I). When properly arranged they make a most instructive series. At one end there is a freely branched, mycelial, non-acid-fast organism, and at the other, one which is bacillary and acid-fast. The extreme mycelial type is represented by No. 585 (Foulerton). Its appearance is characteristic of these species. It forms a thick, leathery, mould-like growth. If discrete, the individual colonies are discoid; if they are confluent, the growth is film-like. It is frequently quite closely adherent to the medium. If the culture is old or the medium dry, a white, powdery appearance shows the presence of true spores. Stained films show the typical branched mycelium, gram positive,

TABLE I.

Morphological and Cultural Characters.

Organism.	Source.	Nature of growth.	Pigment.	Character of mycelium.	Fragmentations.	Gram positive.	Acid-fastness.
S. 585 hominis III	Foulerton (appendix)	Thick and leathery, if old, or dry white efflorescence	Very slight brown, if soluble	Long, branching	Small number	+	o
1	Isolated from infection	Thick and leathery, if old, or dry white efflorescence	Marked brown, if soluble	Long, branching	Small number	+	o
S. pulmonalis	Am. Mus. Nat. Hist. (from lung of cow)	Thick and leathery, if old; slight efflorescence, very mouldy odor	Deep brown, soluble	Long, branching	Small number	+	o
S. bovis	Am. Mus. Nat. Hist. (Parke, Davis & Co. stock)	Less thick and leathery; no white efflorescence	Light brown, soluble	Slender, branching	Marked number	+	o
S. 1276 hominis IV	Foulerton (hand infection)	Thick, leathery; abundant efflorescence	Deep brown, soluble	Long, branching	Infrequent	+	o
S. maduræ	Am. Mus. Nat. Hist. (Kral, 1902)	Thick, mould-like; no efflorescence	No pigment	Long, branching	Moderate	+	o
S. gabritschewsky	Pasteur Institute	Dry, crumbling, heaped up; no efflorescence	Deep brown in growth, soluble; agar almost black	Very short, branching	Very marked, bacillary and coccoid	+	o
S. asteroides	Am. Mus. Nat. Hist., St. Louis Univ.	Dry, discrete granules; no efflorescence	Colorless in young, orange in old culture; indiffusible	Long, slender, branching; much fragmented	Very marked, long, bacillary segments	+	+ (in older cultures, some parts only of mycelium. Appears with orange color.)

TABLE I.—*Continued.*

Organism.	Source.	Nature of growth.	Pigment.	Character of mycelium.	Fragmentations.	Gram positive.	Acid-fastness.
S. nocardii	Pasteur Institute	Dry, flakes, heaped up in masses	Yellowish white; indiffusible	Very short, branched	Very marked, bacillary and coccoid	+	+ (A few fragments in old cultures only.)
S. eppingeri	Pasteur Institute	Dry, granular, heaped up in crumbling masses	Bright orange in older cultures; indiffusible	Very short, infrequent	Excessive, bacillary and coccoid	+	+ (20 or 25 per cent, in old cultures.)
S. capræ	Pasteur Institute	Dry, flakes, heaped up in masses	Pinkish yellow; indiffusible	Very little	Very marked, bacillary	+	+ (A small number in old cultures.)
B. lepræ	Clegg	Discrete masses rather moist	Bright orange; indiffusible	None	Bacillary	+	+ (In all ages, resists acid less.)
B. tuberculosis	Human	Lumpy, rather dry, discrete	Whitish or flesh colored	None	Bacillary	+	+ (In all ages, resists acid strongly.)

more or less fragmented, with many or fewer spores on short lateral hyphæ, with many or fewer threads showing chain sporulation, and with some old ones taking the pink counterstain with a long series of minute, deeply gram positive granules within them, which are the results of chain sporulation (figures 1 and 2). Lessened amounts of the free branching mycelium, consequently less true sporulation, and a concomitant increase in segmentation with bacillary and coccoid forms are the chief differences to be seen in the series (figures 3 to 7), until with *Streptothrix gabritschewsky, Streptothrix eppingeri, Streptothrix nocardii* and *Streptothrix capræ,* the bacillary and coccoid forms predominate to a great extent (figures 5 to 7). For comparison the series ends with the organisms of leprosy (Duval's free grower (18)) and *Bacillus tuberculosis,* which show an almost exclusively bacillary form and no mycelium, but have under certain conditions both branching and thread-like structures, and always a chain sporulation as evidenced by the so called beaded bacilli. They are both gram positive and usually acid-fast (table I).

Pigment formation of two sorts occurs in the cultures. One is brownish, often most marked in the lower surface of the growth, and is soluble, as is shown by its diffusing through the agar and coloring it various shades of brown and black. The other is yellow, pinkish, or bright orange, and is not diffusible, the agar never becoming colored. The soluble pigment appears to belong to the non-acid-fast organisms, the indiffusible to the acid-fast members. Any one of either type may, however, fail to show pigment.

The growth in the mycelial and bacillary types differs, moreover, in general appearance. In the former it is tough, more or less adherent, and in isolated colonies like small blisters on the agar; the growth of the bacillary type is usually dry, mealy, and crumbling, and heaps up on the surface. Separate colonies are like fine grains of sand.

All these organisms are gram positive in cultures if young and active, the fragmentations, spores, and young sprouting mycelium being especially intensely colored. When losing its vegetative state, the mycelium begins to color poorly and ultimately only the trans-

formation products stain, and the faint mycelial outline is indicated only by the counterstain.

Acid-fastness is a character of strikingly variable occurrence. Six of the species studied, showed more or less of this quality: *Streptothrix asteroides, Streptothrix nocardii, Streptothrix caprœ, Streptothrix eppingeri,* and, of course, *Bacillus leprœ* and *Bacillus tuberculosis.* The conditions leading to the development of this property are at present obscure. Why, of a group or series of segments in a mycelial thread, one or more individuals should become acid-fast while its neighbor on each side remains non-acid-fast is unknown (figures 5 to 7). A small percentage of individual organisms in *Streptothrix nocardii,* more in *Streptothrix caprœ,* and still more in *Streptothrix eppingeri* are always acid-fast. In the first two species after some weeks or months of growth, in the last one after three to four days, many acid-fast organisms are produced. *Bacillus leprœ* and *Bacillus tuberculosis* are the most constant and absolute in their acid-fastness, but both show variation in form and tinctorial properties under certain conditions (Foulerton (3), Williams (19), Galli-Valerio (20)). No doubt acid-fastness represents a more resistant state, and fluctuates to a marked degree, being really more a functional than a structural character.

With all these differences there are a few points in which all the members of this series are alike. When grown in bouillon, the medium always remains clear. The growth on solid media is discrete, not smeary and flowing as is the case with many of the bacteria. They are all gram positive, all have granule formation, either in mycelium or its products, as shown in chain sporulation, and, finally, branching organisms and thread-like forms are seen more or less frequently in all species. The literature gives reference to branching tubercle bacilli and actinomyces-like growth of the respective organisms in the tissues of leprosy, glanders, diphtheria, and tuberculosis (Galli-Valerio (20)). This perplexing pleomorphism is readily explicable if we consider these organisms to be bacillary specializations from a streptothrical type, and closely related to this group rather than to the true bacilli.

Of the serological tests which can be applied to any organism, alexin fixation is at once the most accurate and the most specific. It

indicates the change undergone by the body cells in response to the introduction into the system of a bacterial or other protein antigenic substance. There is difference of opinion as to the possibility of its use for quantitative determinations of immune bodies in a serum, at least in so far as varying amounts in any given individual may be indicated. The intensity of fixation gives no evidence as to the amount of antibodies present under these circumstances. However, it seems possible that the results obtained in the present work indicate that alexin fixation can be used as a group reaction, if all the experimental conditions are closely parallel and accurately controlled. Hence generic as well as specific relationships between organisms can be shown, and by applying the test to organisms of debatable or unknown position their true status may be indicated. This is a somewhat different problem, since the serum is, as it were, a fixed quantity and the antigen the variant. The increments of change are larger and more readily recognizable.

In order to carry out such a test with the series of organisms already described, three differentiated and characteristic species were chosen. These were: *Streptothrix 585,* which was mycelial and non-acid-fast; *Streptothrix eppingeri,* which was very slightly mycelial and partly acid-fast; and *Bacillus tuberculosis,* which was non-mycelial and completely acid-fast. Rabbit sera, obtained after immunization with these three organisms, were used in fixation experiments with antigens made from each of the thirteen strains. Preparations of these particular organisms in normal salt suspensions and antiformin preparations proved useless as fixation antigens, but the Besredka method of endotoxin extraction modified by Gay[1] yielded highly satisfactory results. The antigens were made as follows:

1. The cultures were planted on large, flat sided Blake bottles of agar or glycerin agar and incubated at 36° to 37.5° C. until the maximum growth appeared. This varied with the species from five to sixteen days. The lower temperature was more favorable than the higher.

2. The growth was washed off with a small amount of sterile salt solution. In some cases this proved quite difficult owing to the close adherence of the growth to the medium.

3. The saline suspensions were precipitated with equal parts of absolute alcohol and centrifugalized.

[1] Gay, F. P., personal communication.

4. A few cubic centimeters of absolute alcohol were added to the residue to wash the sediment into a sterile evaporating dish. It was then dried in a partial vacuum over sulphuric acid for from twelve to twenty-four hours.

5. The dry residue was scraped off, collected in a sterile dish, and carefully weighed. The necessary amounts of sodium chloride and water were calculated to make it into a 2 per cent. solution in 0.85 per cent. normal salt. The dry salt was added to the residue and the resulting mixture ground together in a sterile mortar for one hour; then the water was added. Finally, 0.5 per cent. carbolic acid was added to the preparation and it was sealed in a tube.

When used these preparations were shaken up and dilutions made of the resulting liquid. They were used in full strength for immunizing the animals.

A preparation of *B. tuberculosis* was made in exactly the same way from the dry residue of a bouillon filtrate obtained from the Cutter Laboratory. This gave a series of antigens analogous in every way and of known strength, so that for any dilution used the exact amount of the original culture might be calculated in milligrams.

The usual method of injecting animals at weekly intervals proved unsatisfactory; they developed severe cachexia, shown by loss of hair and weight, great hypersensitiveness, and skin lesions of a purpuric character. Their condition finally became so bad that they had to be bled. They yielded a serum of little or no potency.

The following method was used: One half a cubic centimeter of the desired antigen was injected into the marginal ear vein of a normal adult rabbit four times at three day intervals. Eight or nine days after the last injection, the animals were bled to death and the resulting serum was inactivated at 56° C. for thirty minutes. The whole process consumed twenty to twenty-one days. This time can probably be still further shortened as Gay and Fitzgerald (21) have recently shown.

The adoption of these methods for the preparation of the antigens and sera has two distinct advantages; first, a potent experimental serum, and second, absolutely standardized antigens. Failure to obtain satisfactory results from fixation experiments after artificial immunization with these organisms is doubtless due to neglect of one or both of these essentials.

TECHNIQUE OF FIXATION EXPERIMENTS.

A constant amount of immune serum was used in all the fixation experiments (0.3 c.c.) with varying dilutions of the antigens. The 2 per cent. solutions of the latter were diluted with salt solution (0.85 per cent.) to 1/10, 1/20, 1/40, 1/80, 1/160, and 1/320 of the original strength, and 1 c.c. of these dilutions was used

for each tube of the experiments. The actual amounts of dry antigenic substance represented in 1 c.c. thus used are as follows:

1/10	dilution = 2.0	mg.
1/20	dilution = 1.0	mg.
1/40	dilution = 0.5	mg.
1/80	dilution = 0.25	mg.
1/160	dilution = 0.125	mg.
1/320	dilution = 0.062	mg.

Exceptions to this rule were made with the antigens of *S. maduræ* and *S. lepræ,* both of which contained so much inhibitory substance that hemolysis did not take place even in the four lower dilutions in the controls with normal serum. In the higher dilutions hemolysis appeared, and when used in 1 per cent. instead of 2 per cent. solutions, the results were distinctive, as indicated in the tables.

Further details of the experiments are as follows:

Antigenic Solutions.—1 c.c. of sodium chloride (0.85 per cent.) dilutions from 1/10 to 1/320 of the 2 per cent. preparations of the different organisms.

Antiserum.—0.3 c.c. (56° C.).

Alexin.—0.1 c.c. of guinea pig serum separated from the clot eighteen to twenty-four hours after bleeding; dilution to 1 c.c. in salt solution, incubated with antigen and antibody at 37° C. for one hour.

Hemolytic System.—1 c.c. of a 5 per cent. suspension of washed sheep blood containing four minimal hemolytic doses of a strong rabbit-anti-sheep serum.

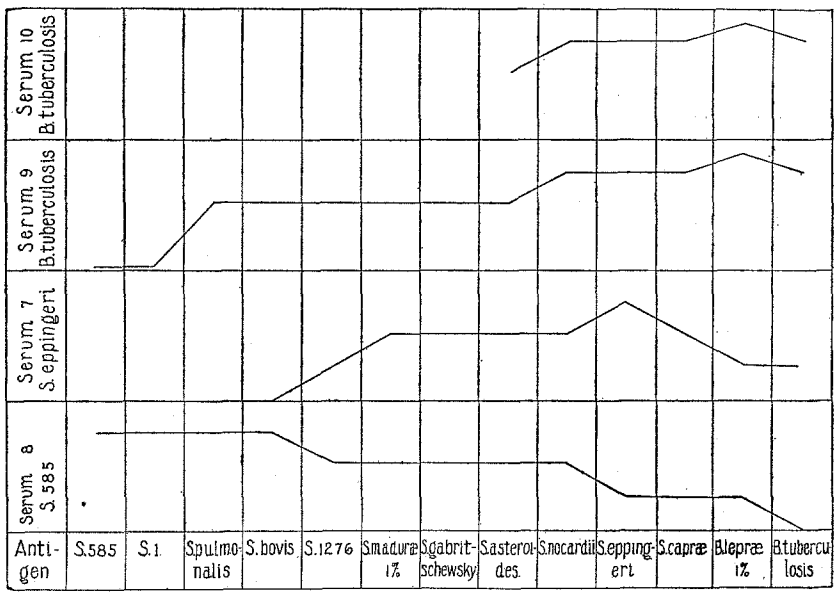
Positive Fixation.—Complete absence of hemolysis after two hours at 37° C. followed by sedimentation over night in the ice box.

Controls.—1/10 and 1/20 dilutions of each antigen plus 0.3 c.c. of inactivated normal rabbit serum. Antiserum with salt replaced the antigen. These should hemolyze completely.

In the experiments carried out with six dilutions, 1/10 to 1/320, three controls were used, 1/20, 1/80, and 1/320.

The results of the experiments are graphically represented in text-figure 1, which is charted from table II. It is seen that the immune serum of *Streptothrix 585* gives decreasing fixation with the various antigens as the bacillary and acid-fast organisms are approached, fixation ceasing to occur with the antigen of *Bacillus tuberculosis.* The immune serum of *Bacillus tuberculosis,* on the other hand, fixes with all the different antigens of the series excepting that of *Streptothrix 585.* The serum of *Streptothrix eppingeri,* the intermediate type, fixes both less widely and less intensely, with three non-acid-fast and six acid-fast species. It shows a distinct though feeble fixation with the antigen of *Bacillus tuberculosis,* but none with the first four mycelial strains.

A second serum, No. 10, immune to *Bacillus tuberculosis,* was used in experiments with the acid-fast organisms only. The antigens were diluted from 1/10 to 1/320 in these tests. They confirmed the findings with the other similar immune serum.



TEXT-FIG. 1. Diagram of fixation experiments.

These results seem to show definitely that a quantitative differentiation between antigenic substances can be made by fixation tests. In this series there are apparently two antigenic substances; one is dominant at the mycelial end. As this substance is gradually reduced, another is substituted and becomes in turn characteristic of the opposite end. In the intermediate members, presumably both substances are present, but neither one is in sufficient amount to cause intense fixation. There is, on the whole, a stronger fixation with the acid-fast members than with the mycelial.

Much (13), Much and Hoessli (22), Deilmann (23), and others, in their work on the specific substances of *Bacillus tuberculosis* and other acid-fast organisms, believe that they are of three kinds, causing three corresponding antibodies to arise in the serum of immu-

TABLE II.

Details of Fixation Experiment.

Antigen.		Serum 8 against *S. 585*, 0.3 c.c.	Serum 7 against *S. eppingeri*, 0.3 c.c.	Serum 9 against *B. tuberculosis*, 0.3 c.c.	Serum 10 against *B. tuberculosis*, 0.3 c.c.	
S. 585	1/80	0[2]	0	0		
	1/40	+	0	0		
	1/20	+	0	0		
	1/10	+	0	0		
S. 1	1/80	0	0	0		
	1/40	+	0	0		
	1/20	+	0	0		
	1/10	+	0	0		
S. pneumonalis	1/80	0	0	0		
	1/40	+	0	0		
	1/20	+	0	+		
	1/10	+	0	+		
S. bovis	1/80	0	0	0		
	1/40	+	0	0		
	1/20	+	0	+		
	1/10	+	0	+		
S. 1276	1/80	0	0	0		
	1/40	0	0	0		
	1/20	±	0	+		
	1/10	+	+	+		
S. maduræ 1 per cent.	1/80	0	0	0		
	1/40	0	0	0		
	1/20	+	+	+		
	1/10	+	+	+		
S. gabritschewsky	1/80	0	0	0		
	1/40	0	0	0		
	1/20	±	±	±		
	1/10	+	+	+		
S. asteroides					1/320	0
					1/160	0
	1/80	0	0	0	1/80	0
	1/40	0	0	0	1/40	0
	1/20	±	+	±	1/20	±
	1/10	+	+	+	1/10	+
S. nocardii					1/320	0
					1/160	0
	1/80	0	0	0	1/80	0
	1/40	0	0	±	1/40	±
	1/20	±	+	+	1/20	+
	1/10	+	+	+	1/10	+

[2] 0 = no fixation (complete hemolysis); ± = partial fixation; + = complete fixation (no hemolysis).

TABLE II.—*Continued.*

Details of Fixation Experiment.

Antigen.		Serum 8 against *S. 585*, 0.3 c.c.	Serum 7 against *S. eppingeri*, 0.3 c.c.	Serum 9 against *B. tuberculosis*, 0.3 c.c.	Serum 10 against *B. tuberculosis*, 0.3 c.c.	
S. eppingeri					1/320	0
					1/160	0
	1/80	0	0	0	1/80	0
	1/40	0	±	±	1/40	±
	1/20	0	+	+	1/20	+
	1/10	+	+	+	1/10	+
S. caprœ.....					1/320	0
					1/160	0
	1/80	0	0	0	1/80	0
	1/40	0	0	±	1/40	+
	1/20	0	+	+	1/20	+
	1/10	+	+	+	1/10	+
B. leprœ 1 per cent. ...					1/320	0
					1/160	0
	1/80	0	0	0	1/80	±
	1/40	0	0	+	1/40	+
	1/20	0	±	+	1/20	+
	1/10	+	+	+	1/10	+
B. tuberculosis...					1/320	0
					1/160	0
	1/80	0	0	0	1/80	0
	1/40	0	0	±	1/40	+
	1/20	0	0	+	1/20	+
	1/10	0	+	+	1/10	+

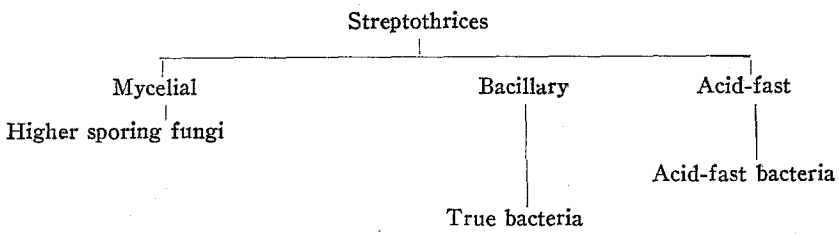
nized animals: a protein, fatty acids, and a neutral fat (tuberculonastin). The last named yields the most intense fixation of the three. However, it has recently been shown by Fitzgerald and Leathes (24) that pure lipoidal substances are incapable of producing antibodies. It is possible that nastin is an impure substance and contains the proteid that is characteristic of the acid-fast organisms and produces fixation with their sera, the mycelial proteid, as we may call it, being reduced to a comparatively small and inconspicuous amount.

However this may be, the essential fact is the possibility of using the fixation test to indicate the biological relations of these organisms. There seems to be no reason why it cannot be equally well applied to other microörganisms of uncertain or unknown relationship, and thus make clear their position. We are here dealing with

a direct means of demonstrating some of the deep and fundamental protoplasmic modifications which make themselves apparent in the changes that we recognize ultimately as making specific and generic characters.

DISCUSSION AND CONCLUSIONS.

It is impossible from any point of view, morphological, biological or serological, to draw a sharp dividing line in this series. The forms change gradually from the mycelial organism to the bacillary, acid-fast organism. It is biologically a group complex and should be so considered. No doubt experiments with a larger series of species would yield results giving a possibility of closer classification and the introduction of some of the forms now in a debatable position, as *Bacillus diphtheriæ* and *Bacillus mallei,* and other organisms, sometimes called, on account of their morphological irregularities, corynebacterium and mycobacterium, would help to show their real relation to both the Streptothrices and the true bacteria. The latter in many ways are acknowledged to be far from primitive; their endospores, flagella, and food habits all indicate a relatively high degree of specialization. Hence it would seem biologically more reasonable to look upon this group of Streptothrices with their variable morphology and close relationships as representing the ancestral type that gave rise to both the higher fungi and true bacteria, and not as being themselves higher bacteria. The various bacteria, other than the acid-fast forms, can readily have arisen from the non-acid-fast bacillary types or even as non-acid-fast specializations of the mixed types. All the various forms shown at present by the bacteria,—cocci, spirilla, bacilli, etc.,—either separate or in chains and masses, are to be recognized in this group, and specializations in one or another line in the past would readily have given rise to the types we consider true bacteria. The processes of evolution have carried them far away from the parent stock and made them into this group. The recognition of this group complex and of the intermediate forms indicates clearly the past history and present relations of these interesting organisms. These relations may be represented by the following scheme.



It is probable that the relation between the acid-fast organisms and the Streptothrices is a closer one than that between the Streptothrices and the bacteria, perhaps close enough to warrant a common genus for both.

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EXPLANATION OF PLATES.

PLATE 8.

FIG. 1. *Streptothrix 585 hominis III* (Foulerton), young culture. (*a*) Actively growing mycelium; (*b*) young sprouting piece; (c) fragmentations.

FIG. 2. *Streptothrix 585 hominis III* (Foulerton), old, dry culture. (*a*) and (*b*) As above; (*c*) true spores attached to spore-bearing hyphæ; (*d*) isolated spores, rod segments, and mycelium undergoing chain sporulation; (*e*) degenerate old mycelium.

PLATE 9.

FIG. 3. *Streptothrix 1276* (probably *hominis IV* (Foulerton)). (*a*) Growing mycelium; (*b*) old mycelium undergoing chain sporulation; (*c*) fragmenting mycelium and its products.

FIG. 4. *Streptothrix pulmonalis.* (*a*) and (*b*) As above; (*c*) young sprouting mycelium.

PLATE 10.

FIG. 5. *Streptothrix gabritschewsky.* (*a*) Fragmentations, coccoid, bacillary, spiral. No marked branching forms as in *S. hominis* and *S. pulmonalis;* (*b*) old mycelium.

FIG. 6. *Streptothrix nocardii,* three months culture. (*a*) Mycelium showing coccoid fragmentation. Very pale staining substance between the deeply blue staining non-acid-fast cocci; (*b*) a string of acid-fast organisms.

FIG. 7. *Streptothrix capræ.* Strongly bacillary with many acid-fast organisms, beaded, practically no mycelium. (*a*) Mycelium showing fragmentation; (*b*) acid-fast individuals.

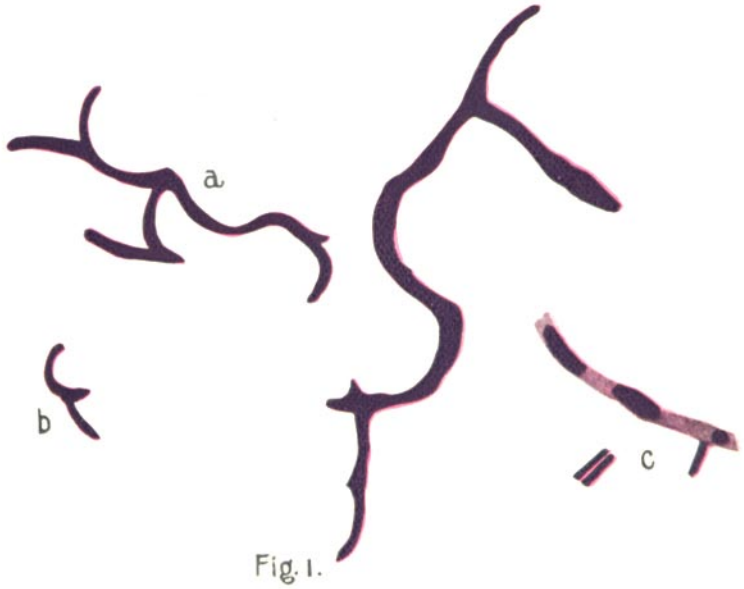


Fig. 1.

Fig. 2.

(CLAYPOLE: Classification of the Streptothrices.)

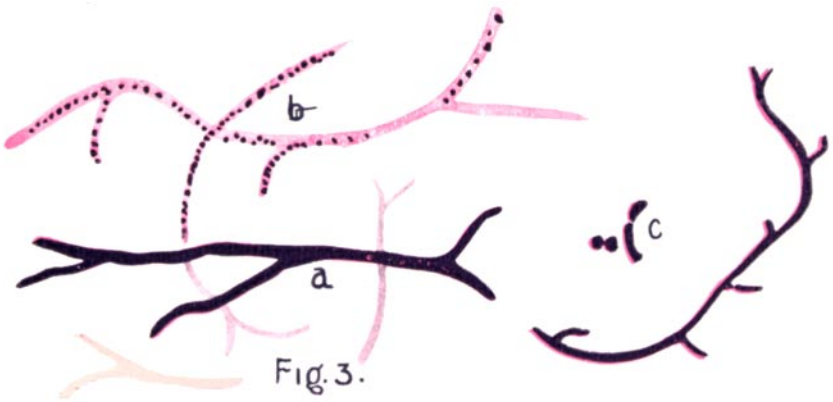
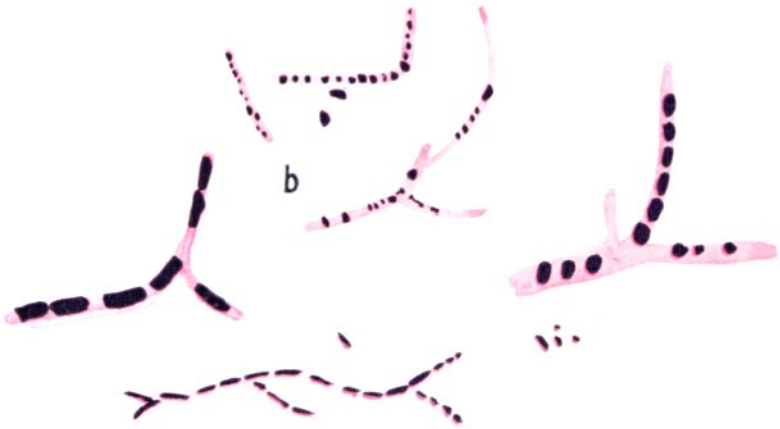


Fig. 3.



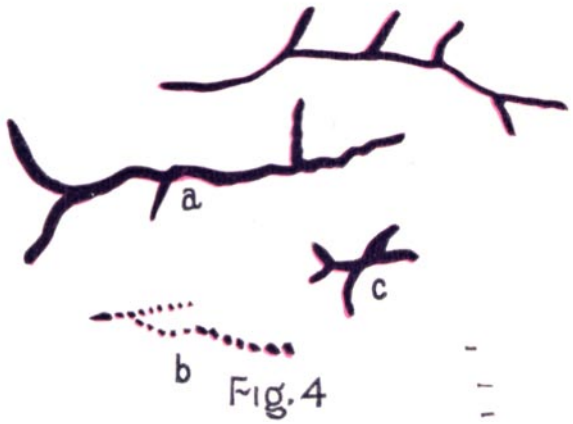


Fig. 4.

(CLAYPOLE: Classification of the Streptothrices.)

Fig. 5

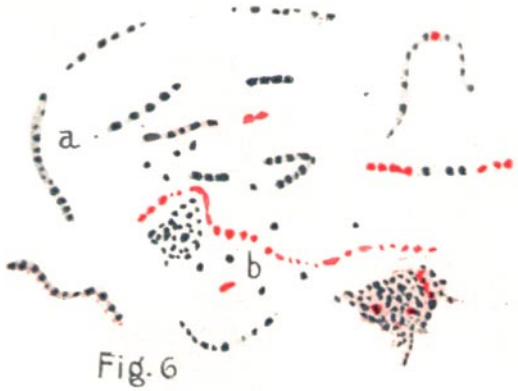


Fig. 6

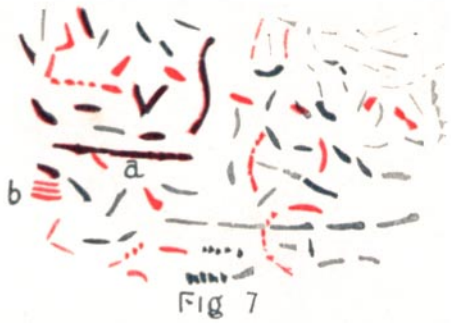


Fig. 7

(CLAYPOLE: Classification of the Streptothrices.)