

A NEW ACID-FAST STREPTOTHRIX, PATHOGENIC TO MAN AND ANIMALS.

(Plate I: Two Figures.)

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THE numerous additions to the group of *Streptothrices* which have been recently made, and the close resemblance which the majority of these newly described forms bear to one another, render it by no means an easy task to assure oneself that a freshly isolated member of this group is one that has not previously been met with and described. A curious fact also, which soon strikes anyone who investigates the literature relating to this group of micro-organisms, is that in very few of these has the particular form described by one author been met with by another. While great interest to the bacteriologist naturally attaches to any new species, whatever its source, the group of those which are pathogenic in their action on man, on animals, or on both, are, *ipso facto*, of more general importance. To this last category belongs the new member of the group which we have isolated from a fatal case of lung disease and pericarditis in man.

While the general morphological resemblance between the numerous forms of *Streptothrix* described is sufficiently clear to allow of no doubt as to their belonging to the same family, the points in which they differ from one another are less marked. These points may be shortly classified into (a) Pathogenicity to man or animals. (b) Cultural characteristics. (c) Staining reactions.

The organism under discussion was detected during life by means

of the last of these criteria, so the staining properties may be referred to in the first instance. In the smaller manuals of Bacteriology as a rule only two organisms, the *Bacillus leprae* and the *Smegma bacillus*, are noted as having the same peculiarity as the *Bacillus tuberculosis* in resisting decolorisation by a 25 per cent. solution of mineral acids after having been stained in hot carbol-fuchsin. This property is shortly designated as "Acid-fast" by German writers and is not uncommonly possessed by the various species of *Streptothrix*. To this acid-fast group the *Streptothrix* we have isolated belongs, and much of the interest at first attaching to it was due to the fact that, in the case from which it was isolated, the clinical diagnosis of pulmonary tuberculosis was thought to be confirmed owing to the appearance in the sputum of acid-fast rods, in many instances closely resembling *B. tuberculosis*. More careful examination however showed points of difference which will be detailed below.

Our knowledge of acid-fast microbes has of late been greatly extended. Nocard (1888) found that the organism causing 'Farcin du Boeuf,' the *Streptothrix bovis*, stained in this manner. The *Streptothrix* isolated by Eppinger (1890), from a diseased condition in man, presented the same characteristic, as did that described by Sabrazès and Rivière (1895). The organisms described by Petri and Rabinowitsch (1897), and the three forms found by Moeller (1897), viz. his 'Mist' Bacillus and Grass Bacilli Nos. 1 and 2, also resisted decolorisation by acids, but have not been found as the cause of disease in man. Pappenheim (1898) has recorded a case of gangrenous lung abscess in which bacilli were found, closely resembling *B. tuberculosis*, but, apparently, he did not succeed in obtaining a culture of the organism. The second organism described by Berestnew (1898) as a Pseudo-Actinomyces, which produced a fatal illness in a shoemaker, was also acid-fast. He also states that, in six cases of actinomycosis in cattle, the clubs in the younger nodules stained well by the method used for *B. tuberculosis*.

The history of the case from which the new *Streptothrix* was isolated was as follows:—

F. E. 26 years, Private 5th Dragoon Guards, belonged to the beleaguered garrison of Ladysmith. He contracted a fever there in January 1900, complicated with dysentery, from which he never completely recovered. On his arrival at Netley in May, 1900, he was evidently dangerously ill, pale, emaciated and suffering from hectic fever. Signs of fluid were found in his right pleural cavity and great enlargement of the liver. He had cough, with expectoration of reddish muco-purulent

sputum. On microscopical examination of the sputum numerous acid-fast rods were found, closely resembling *B. tuberculosis*. At the same time a few thin, segmented branching filaments, also acid-fast, were noted and supposed at the time to be the actinomycotic form of *B. tuberculosis*. There was no infiltration of the skin of the thorax. An exploratory puncture was made in the right pleural cavity and some odourless pus of a chocolate colour and somewhat slimy consistence was removed. Cultures made from this pus yielded the organism in question in nearly pure culture. The resemblance of the pus to that derived from a liver abscess was so striking that it was considered to have possibly arisen from that organ. On the following day Major Dick, R.A.M.C., made two incisions, one in the right posterior axillary line in the 7th interspace, the other in the right nipple line in the 8th interspace, and evacuated a quantity of pus, of the character described above. There was no marked haemorrhage nor discharge of soft brain-like matter, such as has been described in most cases of actinomycosis (Godlee, 1900). The liver was punctured to determine whether the empyema was due to the bursting of a hepatic abscess, with a negative result. No improvement of the general condition followed on the operation. Signs of pericarditis appeared, and the diarrhoea and fever increased and were accompanied by increasing prostration. He died 11 days later on the 21st May, 1900.

At the *post mortem* examination the right pleural cavity was found to be the seat of an advanced empyema. Both layers of the pleura were greatly thickened and coated with a rough pyogenic membrane. Both operation wounds communicated freely with the pleural cavity. The right lung, which was firmly adherent to the diaphragm, was in a condition of chronic broncho-pneumonia, but was free from nodules or cavities. Its weight, combined with that of the liver, with which it was removed 'en masse,' was 7 lb. 10 oz. (3458 grms.). The left lung weighed 2 lbs. (907 grms.) and was studded throughout with small cirrhotic nodules, showing no trace of caseation and differing in many respects from miliary tubercles. The liver was greatly enlarged and somewhat pale on section. On its convex surface it showed a small ecchymosed track, penetrating 1 inch into the right lobe—the side of the exploratory puncture noted above. There were neither fresh abscesses nor cicatrices of old ones. The heart, which, when freed from clot, weighed 12 oz. (340 grms.) showed some hypertrophy of the left ventricle but was otherwise normal. The pericardium was greatly distended, 22 oz. (625 c.cm.) of serum containing numerous flakes of lymph being removed from it. Both layers were thickened and coated with a shaggy deposit of recent lymph.

There was some thickening and pigmentation of the mucous surface of the colon in the neighbourhood of the sigmoid flexure, and a few shallow ulcers with irregular margins.

Cultures were made from the nodules in the left lung, the empyema pus, and from the pericardial fluid, with the result that the *Streptothrix* was again recovered, in pure culture, from the pericardial fluid and also, in conjunction with pyogenic organisms, from the pus and the lung.

Microscopically the *Streptothrix* was seen in scrapings from the pneumonic nodules in the lungs in large quantity, and presented the same characters as were noted in the sputum during life, except that the branching was more luxuriant. Sections of hardened lung tissue showed a chronic pneumonic process with numerous cirrhotic foci and the presence in considerable numbers of acid-fast bodies closely resembling those figured by d'Arrigo. None of the usual appearances of tubercular disease, giant cells, epithelioid cells, or caseation, were found.

The *Streptothrix* occurred in the pus as a fairly open network of long thin threads, segmented and showing lateral branches which came off at right angles. In length, they were seen occasionally to stretch almost across the field of the microscope ($\frac{1}{12}$ " oil immersion). The width of the threads averaged about 0.5μ and was fairly uniform throughout without any appearance of clubs or spore formation. The threads stained well with all basic aniline dyes and retained Gram's stain. When treated by the Ziehl-Neelsen Carbol-Fuchsin and decolorised by 25 per cent. sulphuric acid and alcohol they remained deeply stained.

Behaviour in Cultures.

Very slight growth, if any, occurred at room temperature. On *Gelatine Plates*, after 5—7 days' incubation at 22° C., small white circular colonies appeared on or close to the surface. On examination by a low power these were seen to resemble small hemispherical balls of cotton-wool, snow-white by reflected light and centrally tinged yellowish-brown by transmitted light. No liquefaction of the medium occurred after three weeks' incubation, and the colonies ceased to enlarge after 8—10 days, attaining a maximum diameter of about 1 mm. In *Gelatine-stab* cultures a growth appeared on the surface similar to that on plates with a slight expansion for a short distance down the needle track.

On *Agar* its growth is rapid. After 36—48 hours a snow-white dry powdery growth appears which a few days later takes on a delicate pale coral-pink tinge where the growth is thickest. The pink tint is very

constant and appears, after a varying period, on cultures in nearly all media where the organism is freely exposed to the air. The water of condensation remains clear. A few rugged granules may appear on the thicker portion of the agar slope, but they show no tendency to run together into a film, and attain a maximum diameter of about 1 mm. in a week. The growth is, in cultures of less than 10 days' incubation, easily removable by the needle from the surface of the agar, and, even in old cultures, never adheres as firmly to the medium as do so many other forms of *Streptothrix*. (See Plate I, fig. 1.)

In ordinary nutrient *Broth*, after 24—36 hours' incubation at 37° C., delicate white specks are seen floating on the surface, and this appearance, likened by Besson to water-lily leaves, is common to the majority of the *Streptothrices*. These specks increase slowly in size and become more or less spherical in shape, the portion above the surface of the fluid being dry and tinged coral-pink, while that below appears woolly and white. The growth, while never forming a felted, coherent scum, may extend up the sides of the tube, but, as a rule, the separate rosettes or colonies, after 1—2 weeks, sink to the bottom of the tube, retaining their shape and individuality. The broth throughout remains clear and odourless and its alkaline reaction unaltered. No indol is produced. The addition of a little sterile synovial fluid to ordinary broth enhanced the growth of the organism and increased its microscopical resemblance to the form in which it was originally noted in the empyema pus.

On *Potato*, which, whether glycerinated or not, forms a very favourable medium, a copious dry white growth occurs in 48 hours, giving the appearance of a splash of plaster of Paris. The growth never extends more than 2—3 mm. from the needle track, and though becoming granular and warty with age shows no tendency to wrinkle on the surface. The coral-pink tint develops early and is noticeable on the 3rd or 4th day, especially when the superficial white powdery coating is removed by the needle.

In *Milk*, a surface growth of isolated rosettes occurs, similar to that in broth. No clotting occurs, but in old cultures the milk is digested and a deposit of rosettes and casein takes place. The reaction of the milk is alkaline.

Solidified blood serum. No growth.

In Sterile Tap-water. After four days a somewhat scanty growth appeared on the surface resembling that in broth, but the floating colonies sank to the bottom of the fluid in a shorter time.

Conditions of Growth.

While free development occurs at 22° C. and optimum growth at 37° C., temperatures much higher than this have failed to devitalize it; indeed, it might almost be included in the group of thermophilic bacteria. Growth took place on potato after 48 hours at 46.5° C. but none after incubation at 50° C. Heating for five minutes at a temperature of 75° C. failed to destroy it, though its subsequent cultivation was much delayed. Twenty minutes exposure to 75° C. or momentary exposure to 100° C. destroyed it.

Under conditions of anaerobiosis by displacement of Oxygen by Hydrogen or CO₂, no development took place.

It retains its vitality for a long time. A dried-up agar culture, nine months old, was successfully transplanted.

Morphology of the Streptothrix in Cultures.

While careful examinations and comparison of the character of growth in the various culture-media disclosed numerous slight differences, it will suffice to indicate the features common to them all, only indicating the chief variations. (See Plate I, fig. 2.)

The *Streptothrix* occurs, like most of the members of the group, in two forms, a fine branching network of mycelial threads, and a so-called 'streptococcic' form caused by the breaking up of the terminal threads into a series of small oval segments, whose exact nature has not yet been determined. These latter may, for purposes of description, be termed arthrospores but, though they differ from true spores in their origin, being clearly due to a segmentation of the endo-capsular contents of the original thread, it appears to us probable that they function as true spores and that it is to their abundant formation under suitable conditions that the longevity of the organism is due.

Briefly, on dry media, the threads rapidly break up into arthrospores and these form the dry white powder which overlies the mass of the culture. On the other hand, in fluid media, while the aerial portion of the floating island is largely composed of these arthrospores, the portion below the surface of the fluid consists chiefly of the network of branching threads in which, although segmentation of the threads may be observed, the further development of the segments into arthrospores is but rarely seen.

The acid-fast nature of these two forms of the organism varies with the age of the culture. Klein and Moeller have noted similar features

in old cultures of Tubercle and Grass Bacillus II respectively. When young, both threads and arthrospores retain the fuchsin intensely, later, by degrees, the threads lose this property and, in old cultures, they may become completely decolorised. On the other hand the arthrospores remain acid-fast for as long as we have had them under observation. The same remark applies to Gram's staining, the threads with age becoming decolorised while the arthrospores retain the stain. After growing for some months in the medium to which sterile synovial fluid had been added, dark, almost black beads were noted in the length of the threads on staining by Weigert's method, similar to those arthrospores of the tubercle bacillus figured by Coppen Jones (1895), these beads occasionally were found at the point at which a lateral branch was given off from the main thread. Many stages of the segmentation of threads into arthrospores have been noted in cultures from different media and of different age, from a chain of rods resembling a small chain of *B. anthracis* to a perfect streptococcal form, in some of which a trace of the membrane binding them together could still be detected. The arthrospores when fully developed and free are of fairly uniform size and oval in shape, but do not, when unstained, possess the high refrangibility of bacterial spores.

All forms of the *Streptothrix* are non-mobile, and nothing approaching clubs or involution forms has been noted even in old cultures.

Pathogenicity.

Intraperitoneal inoculation of guinea-pigs caused their death in from five to six weeks. Large collections of caseous matter were found, matting together the omentum and small intestine. Metastatic deposits of like nature were found behind the peritoneum, in the diaphragm and in the testes. When inoculated hypodermically under the skin of the thigh of guinea-pigs, a similar collection of caseous matter formed, breaking down into an ulcer which gradually healed. The presence of the organism, chiefly in the form of threads, was in each case observed in this caseous matter, while in the case of the peritoneal collection the *Streptothrix* was recovered in pure culture.

Comparison of the above cultural and other characteristics of this organism with the published accounts of those of the great majority of other *Streptothrices* will show at once wide differences. There are, however, others in which the resemblance is closer, and it may be well to refer briefly to these, indicating the points of difference between them and the new *Streptothrix*.

The *Streptothrix* isolated by Eppinger (1890) from a brain abscess, while not unlike morphologically, differs from ours by growing to some extent anaerobically, by its characteristic growth in broth—dense surface layers of feltwork which sink to the bottom and are renewed again and again—, and also by the orange tint which all its cultures take on. A culture of this form obtained from Král's laboratory, while growing well on ordinary media, refused to grow in sterile water.

The *Streptothrix* of Sabrazès and Rivière (1895) grew only anaerobically and is thus excluded.

Berestnew's 2nd *Pseudo-Actinomyces* (1890) grew under anaerobic conditions. Gelatine and potato inoculated with it remained sterile. Broth cultures had a foetid smell. It was not pathogenic to guinea-pigs and survived but a few generations.

Nocard's *Streptothrix bovis* (1888) forms a dry membrane on agar and a scaly curdy layer on potato. It liquefies gelatine slowly and shows no apparent growth in milk.

Moeller's Mist Bacillus and his Grass Bacillus 1, or 'Timothy Grass Bacillus' are closer allied to *B. tuberculosis* than to ours. They produce clouding in broth and a bright yellow or orange colour in the various media.

Moeller's Grass Bacillus 2 produces a soft and moist yellow growth on agar and renders milk acid.

The *Streptothrix* isolated by Dean (1900) was not acid-fast and did not grow on potato or gelatine.

The forms recorded by Buchholtz (1897) and Flexner (1898) as occurring in man presented many points of resemblance to that under discussion, but in neither case were they successful in cultivating the organism.

DESCRIPTION OF PLATE.

PLATE I.

Fig. I. Photograph of a pure culture of the *Streptothrix* on agar, after one week's incubation at 37° C.

The individual colonies will be noted to be fairly uniform in size and shape, and to show no tendency to coalesce or to wrinkle on the surface. The colonies are snow-white unless touched with a needle, in which case the characteristic coral pink colour will be found underlying the superficial layer of white 'dust,' formed by the arthrospores.

Fig. II. Film preparation of the *Streptothrix*, from a pure culture in peptone broth, one month old. Stained in warm carbol-fuchsin and treated with 25 per cent.

sulphuric acid and absolute alcohol. Microphotograph made with Zeiss Apochr. 2 mm. N. A. 1.4 and P. Oc. 3 Magnification, 1000 diameters.

The Streptothrix threads, showing lateral ramifications and various stages of segmentation, retain the stain with varying degrees of intensity. The arthrospores, uniformly "acid-fast," are seen in various aggregations, from the streptococcal forms, produced by direct segmentation of one of the threads of the Streptothrix, to the free and isolated arthrospores, nearly uniform in size and oval in shape.

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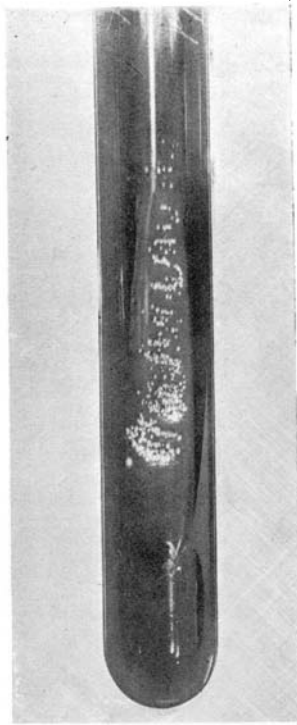


FIGURE 1.

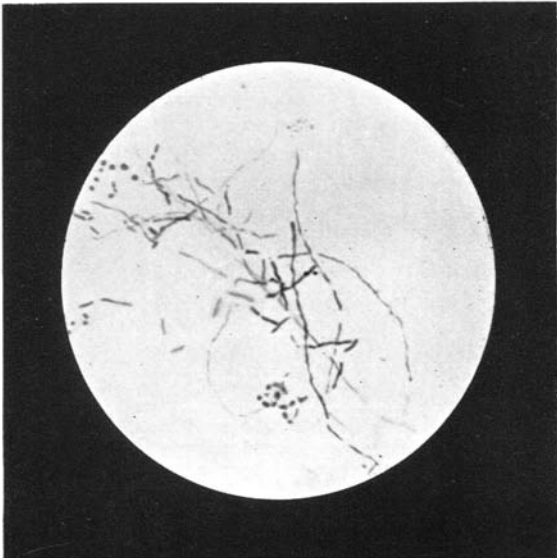


FIGURE 2.