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The Acid-Fast Bacteria

Their Resemblance to and Differentiation from The Tubercle Bacillus.

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D. Sc. 1903.

ILLUSTRATIONS.

I am indebted to my friend Dr. Dixon for his skill and kindness in taking the microphotographs of the pseudo- and genuine tubercle Bacilli. These are all from preparations I have made and are subjected to exactly the same magnification.

York House,
Poole Road,
Bournemouth.

Nov. 28. 02.

This is to certify that the Thesis entitled "The Acid-fast Bacteria" submitted for the D.Sc Pub. Health is the record of original work undertaken and done entirely by myself.

Alfred Charles Cole
M.D. B.Sc.

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The Acid-fast Bacteria.

Their Resemblance to and Differentiation from The Tubercle Bacillus.

The discovery within the last few years of micro-organisms resembling the tubricle bacillus is of the greatest interest to the bacteriologist, and of vital importance to the physician and hygienist.

A few years ago, every acid-fast organism was either the bacillus of tuberculosis or of leprosy, and still later it was shown that in the secretions of the normal skin, especially in the anal-genital region, there existed another organism - the bacillus of smegma, which very closely resembled that of tubricle in its general morphology and particularly in its acid-fast character.

The power of resisting decolourisation by mineral acids after being deeply stained rendered the recognition of Koch's bacillus by means of microscopic examination easy

and certain to all. The bacillus of leprosy is so infrequent at least in this country that its differentiation from the bacillus of tuberculosis was not of material importance.

To these two bacilli - that of leprosy and smegma, many new acid resisting organisms closely resembling and easily mistaken for those of genuine tuberculosis, have been added.

Möller, Petri, Rabinowitsch, Lubarsch and others have found several apparently different species of bacilli, which are not only closely like the tubercle bacillus in form shape and size but which retain the stain when after being coloured according to Ziehl-Neelsen's method they are subjected to the action of acids and alcohol.

They further resemble the true bacillus of tubercle, in that, when inoculated into animals, they produce nodular or tuberculous-like growths.

These acid-resisting or acid-fast organisms are very widely distributed in nature - They have been found with alarming frequency in butter, milk and cheese. Rabinowitsch found them in 28.7%

of samples of butter examined in Berlin and Philadelphia. They have been met with in the secretions and excretions of many herbivora, and in normal and pathological secretions in man. They have been seen in tonsillar exudations, in caries of the teeth, and in the sputum of cases of non-tubercular abscess and gangrene of the lungs.

Möller has found them in grasses, hay pollen, dust of the stable, and in the dung of cattle.

It would have been somewhat surprising if only the bacillus of tubricle and leprosy possessed this acid and alcohol resisting power, and even Koch[1] in 1884 stated that "it was not improbable that in time other bacteria may be discovered, which have the same staining properties as the tubercle bacilli."

Lubarsch, Möller, Bullock and many others have stated that the microscopical examination is now not sufficient for the diagnosis of tubercle. The last named observer* says "it would now appear, that there are quite a number of bacilli which are as acid-fast to acids and alcohol as the genuine tubercle bacillus, and it must

* Rep. No 10 - page 448

be acknowledged that this discovery has given a rude shake to the belief that the microscopic examination of the tubercle bacillus is in itself sufficient to establish a diagnosis of tuberculosis"

Even in pure cultures, I can most emphatically say that many of these bacilli - not all - are, even with the finest lens, indistinguishable from the genuine organism of tuberculosis. I have carefully examined these bacteria in pure cultures, and in their natural condition, after subjecting them to exactly the same method of staining, with a magnification of 2250 diameters i.e. by means of an apo-chromatic 1/12 inch objective with No 18 eyepiece (compensation). As a result of such an examination I can find some differences between the majority of the pseudo- and genuine tubricle bacilli, but here and there are organisms, between which as far as I can see, there is no practical distinction. If this difficulty exists when we are dealing with a more or less pure culture, and can compare them side by side under exactly the same conditions, it is evident that an occasional acid-fast bacillus

in urine, sputum, or milk is in many cases absolutely indistinguishable from Koch's bacillus. One has further to remember that the genuine tubercle bacillus varies very considerably in its appearances in the same culture or in the same specimen of sputum. The following photograph of the better known acid fast organisms will bear out this statement.

My attention was directed to these tubercle like organisms, by my having found acid-fast bacilli resembling tubercle, in the urine of two patients. I subjected the films after treating with acid to the action of alcohol, as this is generally advised as a means of eliminating the smegma bacillus. In both I diagnosed a tubercular lesion. In one case this was confirmed by two leading English pathologist, but was disproved by a continental bacteriologist. In the second case Prof. Sims Woodhead kindly inoculated guinea pigs with the urine, with absolutely negative results.

I have therefore gathered from all available sources as much information as possible on these acid resisting organisms,

and have grown many of them, and by means of pure cultures have ascertained how far they resemble Koch's bacillus of tuberculosis.

With a view of finding a differential method of staining, I have further made a series of experiments into the degree of resistance each species has to the ordinary decolourising agents Eg. acids, alcohol and acid alcohol etc, and as a result have found what I think to be a reliable method of differentiating all acid-resisting pseudo tubercle bacilli from the genuine bacillus of tuberculosis.

In these pages I will give an account of the following organisms:-

i) The bacillus of tuberculosis (human) and its modifications
ii) The tubercle bacillus of birds and cold blooded animals
iii) The bacillus of leprosy.
iv. The bacillus of Smegma.
v. The Timothy grass Bacillus (Möller).
vi. The grass " Bacillus (Möller).
vii. The mist or dung Bacillus (Möller).
viii. The butter bacillus (Petri - Rabinowitsch).
ix. Acid fast bacteria found in secretions of Human lungs
x. Various acid-fast bacilli found in Roux of

Birt and Leishman, of Eppinger and Nocard.

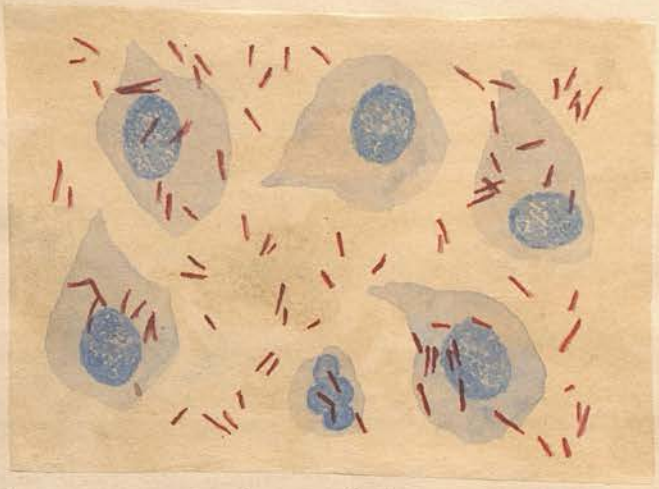
I will then describe what differences I can find between them; and lastly the results of my enquiry into their power of resisting acids, alcohol, etc.

The Bacillus of Tuberculosis and its variations.

As the tubercle bacillus is taken as the standard of the acid- and alcohol-fast organisms, I will briefly mention its most important characteristics, and the chief modifications which it can undergo under varying surrounding conditions.

Koch[1] described his organism as "invariably appearing in the form of small rods of the length of one quarter to one half the diameter of a red blood corpuscle (1.5 to 4 μ); although their length varies, their breadth is pretty constant, provided that the same method of staining is used. The tubercle bacilli are not as a rule quite straight rods: they usually show slight bends or breaks, and often a gentle curve, which may increase in the longest forms to such an extent, as to reach the 1st stage of a corkscrew structure."

Tubercle Bacilli in sputum (from Virchow)
× about 1000.



Bacillus of Tubercle. (Baumgarten)
From Macé. × 1500.

He also in his original description spoke of them as containing oval spores - two to six being present in a single bacillus.

The actual measurement of the bacillus varies considerably. According to Lehmann and Neumann② they are 1.5 to 4 μ long and only 0.4 thick, and according to Macé③ 1.5 to 3.5 μ long and having generally a width of 0.3 μ. The latter points out that the width is much more uniform than the length, and that in preparations stained by Koch's method they usually appear a little thinner than by Ehrlich's.

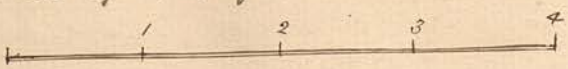
They may be uniformly stained or present small uncoloured spots along their course, with darkly stained parts between. The latter were at first regarded as true spores, but most authorities, although far from being agreed, are of the opinion that no true spores are to be found in the tubercle.

These characters as regards shape and size are by no means constant, either in the organisms met with in cultures or in sputum. Sometimes the bacilli are very short, according to Macé little longer than broad, at other times they are much

② loc. cit page 411.

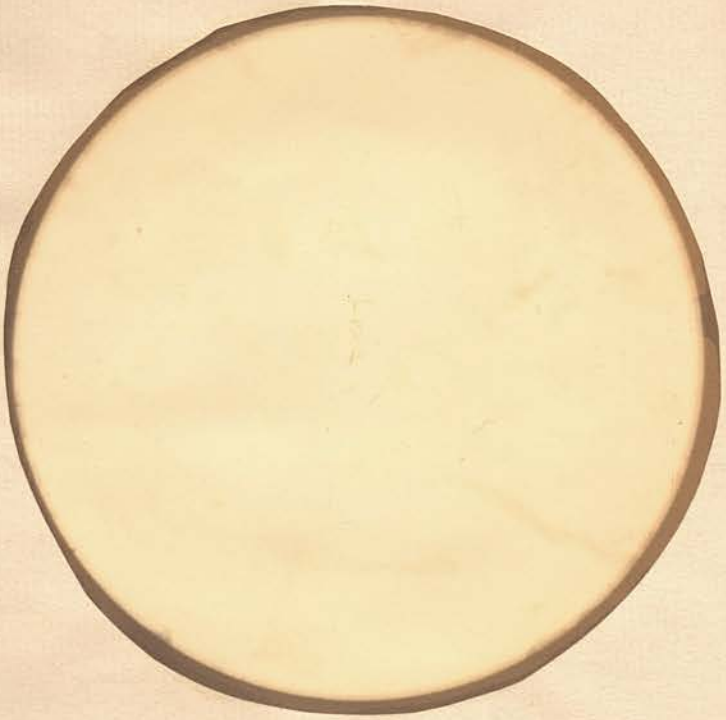
③ loc. cit page 505

Scale of Magnification

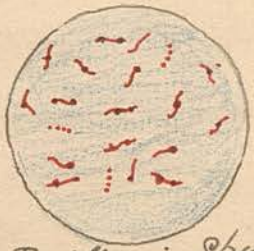


Each division = 1/40 millimetre or 25 μ.

Tubercle Bacilli Human - Microphot'
Culture on potatos.



Tubercle Bacilli in Sputum
Stained by Ziehl Nielsen Method.



Tubercle Bacilli in Sputum.
Most of the bacilli contain
one or more nodules.

larger and may be swollen or clubbed at their extremities.

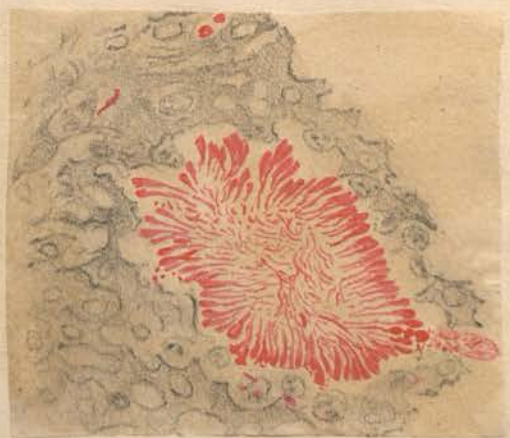
Metschnikoff, Nocard, Roux, Babes, Klein and others have found giant forms, filament and thread forms and true branched forms. These more or less exceptional forms are met with chiefly in old cultures, but also occasionally in sputum. Möller has seen true branching bacilli in tubercular sputum.

My own experience gathered from the examination of a very large number of phthisical sputa is that the most common form met with is the uniformly stained rod which varies somewhat in size, that the next in frequency is the beaded variety.

B. Fränkel has stated that the tubercle bacilli in the more florid forms of tuberculosis took the form of short rods.

In the sputum of one case, I found that many of the bacilli had large nodules, usually one sometimes two or three, situated generally in the middle, sometimes towards the end of the bacillus. These stained very deeply with Ziehl-Nelsen's method, and were considerably wider than the body of the bacillus in which they lay. I have seen these

Clubbed and branched forms of Tubercle Bacilli
after Metchnikoff.



Tubercular granulations of the Meninges of a Rabbit.
×1000. after Babes. Showing actinomyces
form of the tubercle bacilli.

or forms somewhat like them, very frequently in old cultures of human tubercle bacilli.

I have occasionally, though rarely, met with indications of branching forms in sputum.

Metchnikoff[4] who found the long filamentous forms in sputum and in the splenic pulp of tubercular birds, considered that they did not represent evidence of degeneration, but that in all probability only a stage in the developmental cycle of a filamentous fungus.

Hayo Bruns even thought that the aberrant forms belonged to the saprophytic vegetations of an organism which appeared in the form of rods in the parasitic stage.

Coppen Jones[5] as a result of his researches, concluded that in tissues and secretions, the organism occurs as rods, which reproduce by fission, that occasionally in sputum, and always in old cultures, filamentous forms showing true dichotomous branching occur, and these are found on the surface of the medium, whilst in the depths they are only rods. The rods he thinks do not contain true endospores.

Babes and Levaditi[6] found that by injecting rabbits with cultures under the dura mater that in

the infected areas. only rod forms could be found during the first three weeks, but at the end of the fourth week, radiated forms consisting of elongated branched rods with clubbed ends were met with.

Friedrich[6] and Nösske found these clubbed actinomycotic forms, by injecting tubercle bacilli into the left ventricle of rabbits, and unlike other observers, they obtained the best developed radiated forms by the injection of the youngest and most virulent cultures, a fact which is adverse to the theory that these are retrograde forms.

Schultze[8] also found that virulent cultures produced the actinomycotic forms, and concludes that they do not represent evidence of attenuation of the parasite.

Lubarsch[9], as we shall see later, obtained similar ray forms of growths by ~~in~~oculation with the pseudo-tubercle bacilli, notably Möller's Timothy Bacillus, grass ii Bacillus and mist or dung bacillus.

From these facts it seems natural that the tubercle and also the pseudo-tubercle bacilli should be classed among the higher fungi, the actinomyces. In favour of this is the fact that one of the characteristics of the actinomyces is the formation of nodular or

granulomatous inflammations.

The cultivation of the tubricle bacillus presented at first considerable difficulty. Koch used blood serum. Nocard and Roux showed that the addition of glycerine to various media especially to agar facilitates the growth.

"In all cases the isolation of the first strains of tubricle bacilli is not easy, nor is it always possible in a given case. Apparently the main difficulty depends on the slow growth of the organism, so that if other and more rapidly growing bacteria are present - and they frequently are - they may overgrow the medium and render it useless, before the tubricle bacillus has begun its first division." Bullock.[11]

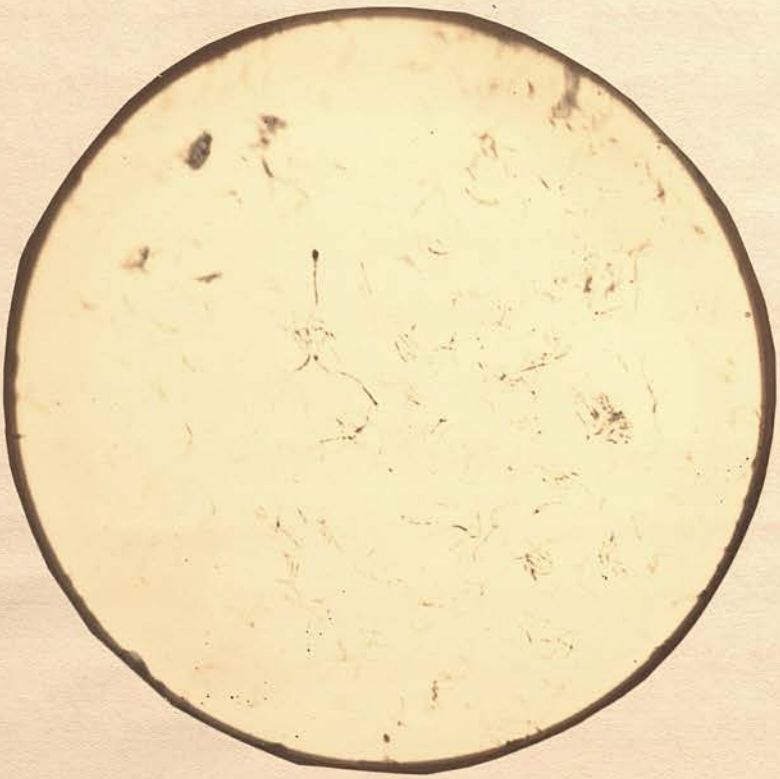
[11] loc. cit. page 494.

Pawlowsky found that glycerinated potatoes in sealed tubes, formed a good medium, and this has since been largely used.

Glycerine potato - agar or bouillon was recommended by Labinski.

Hesse (1899) found that 0.5% of "Nahrstoff Heyden" (a soluble albumin) with 3% glycerine gave rapid growths from tubercular sputum in 1 to 3 days, and according to Bullock and Fränkel this is one of the best media.

The tubricle bacillus grows best at



Tubercle Bacilli - old culture. Microphoto taken from one of Dr. Arthur Ransome's F.R.S. Showing cubbed, and branched, and giant forms.

the temperature of the human body 37°C. The minimal and maximal limit being 29-42°C, therefore probably it does not thrive well outside the human body.

Czaplewiski ⑧ however found that he could get a growing culture of tubercle bacilli to show signs of an increased growth, at the temperature of the room.

Through the kindness of my friend Dr. Arthur Ransome F.R.S, I was enabled to watch many of his experiments on the growth of the tubercle bacilli on various media and also in condensed breath. He succeeded in obtaining growths at the temperature of the laboratory - about 20°C.

Möller, by passing tubercle bacilli through a blind worm, was able to grow them @ 20°C.

The characteristic staining reaction of the tubercle bacillus depends on the fact, that the organism is not easily stained, but when stained, resists decolourisation by mineral and organic acids.

Koch originally succeeded by the addition of alkali, in staining it, but this method is not used now.

Generally speaking the tubercle bacillus is best stained by using a warm solution of a

strong basic aniline dyes eg fuchsin or gentian violet, combined with a mordant such as aniline water or carbolic acid, the usual combination being aniline gentian violet (Koch Ehrlich) or more generally carbolic-fuchsin (Ziehl Neelsen).

Preparations so stained, are not decolorised by the use of 33% nitric acid or 25% sulphuric.

It has been proved that a watery solution of a basic dye, if used hot, can stain the bacillus, but the colour is only partially fast. Bullock (loc. cit. page 499) states that when stained in this manner, the bacilli are decolourised in 1½ hours by a solution of sodium sulphite, whilst if aniline water solution of the dye has been used, twenty four hours immersion in sodium sulphite is borne without effect. Further action of the sodium sulphite produces slow and unequal decolourisation, so that parts of the bacillus - oval forms, situated mostly at the poles - retain the stain, and are sharply demarcated. These egg like bodies, quoting Ehrlich, he says may retain the stain under the influence of sodium sulphite for 8 to 10 days.

Ehrlich was of the opinion that the acid fastness of the tubercle bacillus

was due to the presence of some substance surrounding the actual capsule, which is ~~imperm~~ -iable for aniline, alkalies etc, but impermiable for acids.

Koch[1] himself in 1884 as already mentioned stated that "it was not improbable that in time other bacteria may be discovered which have the same staining properties as the tubercle bacillus." This is now known to be the case.

In addition to the various other acid-fast organisms to be described later, it would be well to mention, that some structures other than bacteria are acid-fast. Amongst others the outer layer of the epidermis, certain layers the capsule of the coccidium oviforme, the ova of tape worms (Bullock)

According to Czaplewski, certain keratinised cells and the nuclei of mast-cells resist partial decolorization.

The acid fast power of the tubercle bacillus was thought to be due to the presence of fat in the organism, and Doroet recommended the use of Sudan III, a fat stain, as a means of identifying the bacillus.

Aronson[2] in 1898 definitely showed that the "saure festigkeit" is due to the presence of

a substance of the nature of wax. If the bacilli are treated with a mixture of alcohol and ether, (Nikroff's fluid) they still retain their power of resisting acids, but if hydrochloric acid is added to the mixture, they are no longer acid-fast. (Bullock).

Aronson and Weyl found that the substance extracted from the bodies of the bacilli by treating them with boiling xylol, chloroform, benzine is very markedly acid-fast.

Borrel found that after removing, by the prolonged action of warm xylol, the wax like substance, the tubercle bacilli had lost their acid and alcohol resisting power, although they were still capable of producing disease.

Removal of fat does not affect either the form or staining reaction of the bacilli.

McLeod and Bullock have isolated this waxlike substance, both from the tubercle and timothy grass Bacillus.

Bullock,[11] as a result of his experiments, draws the following conclusions:-

That the tubercle bacillus though usually in the form of rods may be filamentous, clubbed or like the actinomyces.

That it is difficult to grow, is not adapted

[11] loc. cit. page 502.

for a saprophytic existence, and requires high temperature for its growth. Not under all conditions it is acid fast, due to the presence of a waxlike substance.

Klein[19] and Marmorek[20] have however found that these quite young tubercle bacilli are not resistant to acids and alcohol.

The latter thinks that this is due to the fact, that the young bacillus are not covered with the fatty or waxy envelope, which prevents the ordinary basic pigments easily coming in contact with the bacillus, and which when stained, prevents acids and alcohol from decolourising them.

Klein suggests that the chemical substance which are ordinarily present in tubercle bacilli rendering them resistant to acids, is absent from very young bacilli

Avian Tubercle Bacilli.

In the tubercular disease of birds, bacilli are found which correspond in their morphological characters with and staining reactions, with those in mammals, but differ

in methods of culture and on experimental inoculations. These organisms were first distinguished from mammalian tubercle bacilli by Maffucci[13]

Koch[14] says that both the mammalian and avian tubercle bacilli, are of one and the same kind, as regards nutritive media. He succeeded in getting the tubercle bacillus of mammals acclimatized to a higher temperature, and on some media obtained similar cultures, but he was unable to transfer one to the other as regards their pathogenesis. In guinea pigs by injecting avian tubercle bacilli he induced a general tuberculosis, but cultures taken from this animal were not identical with those of avian tubercle.

The bacilli of avian tubercle must be regarded as only a form of the bacilli of mammal tubercle, which have become accustomed to the higher temperature of birds, but which are also occasionally pathogenic for other animals.

They require a higher temperature for their growth - 42°C - or a minimum and maximum of 35 & 45°C. Unlike the human tubercle bacillus, which does not grow above 42°C - the bacillus of fowl tubercle grows well and does

not lose its virulence at 43°C. (Straus and Gamaleia).

Cultures of bird tubercle have generally a damper and smoother growth on artificial media but exceptions to this are not infrequent.

Lubarsch[1] recognises three forms of growth:- [1] loc. cit page 188.

i. Damp, smooth and easily disintegrated colonies of a slimy consistence.

ii. Dry wrinkled skins which are not easily rubbed apart.

iii. Cultures whist are indistinguishable from those of mammalian tubercle.

He remarks that in one and the same culture different forms of growth may appear by further cultivation.

Kruse[2] found that cultures which at first resembled closely those of mammal tubercle bacilli became damper and softer later, whilst Lubarsch has frequently noticed the reverse, viz that colonies which were at first damp, became under further cultivation especially on agar-agar, drier and wrinkled.

In such cultures he met with true branching and large club shaped forms.

Lubarsch points out that when injected into guinea pigs, after considerable time had

elapsed, the bacilli could be found in the infected areas, arranged in a radial manner, as is the case with the other pseudo-tubercle bacilli.

Tubercle Bacilli of Cold-blooded Animals.

Tuberculosis in cold blooded animals is to be regarded as a modification of tuberculosis in mammals. (Möller)

Dubard and Bataillon[15] cultivated an organism resembling the tubricle bacillus, from a tumour of a carp, which is acid-fast, forms branches, and grows at a temperature of 23 to 25°C with a minimum of 12°C.

They proved that this bacillus is the tubricle bacillus acclimatised to cold blooded animals, by ~~in~~oculating and feeding fish and frogs with cultures of human and avian tubricle, and from the organs of such fish, the bacillus tubricle piscicola was obtained.

Bataillon and Terre succeeded in infecting mammals and birds, by passing tubricle bacilli through frogs at the temperature of the room.

Lubarsch[15] found that the tubricle bacilli of fishes produced pathologically about the same results, as the tubricle bacilli from blind worms and only once did he find the organism arranged in a radial manner.

[15] loc. cit. page 195.

It was also able to modify the tubricle bacillus of mammals by passage through frogs, so that they grew at a temperature of 28 to 30°C.

Möller[16] isolated from the spleen of a blind worm, which had one year previously been infected with human tubricular sputum, cultivations of tubricle which flourished at 20°C. Cultures of these, Lubarsch states, instead of being dry and crumbly, were damp with a shiny white surface.

[16] loc. cit. page 14

They do not grow at a temperature of 28 to 37°C, but grew best at 22°C. Morphologically they are indistinguishable from tubricle bacilli and cultures according to Möller resemble those of bird tubricalosis.

They cannot be ~~inoculated~~ inoculated into rabbits.

The Leprosy Bacillus.

Discovered by Armauer Hansen (22) 1877, and more fully described later by Neisser, the bacillus of leprosy has long been known as an acid fast bacillus, resembling the bacillus of tubercle.

They are usually thin rods, measuring about 5 to 6μ long and 3μ broad, generally slightly curved. When stained they show either a uniform or beaded appearance, darkly stained parts alternating with unstained points. They often appear tapered, but sometimes at one or both extremities, club shaped swellings are seen.

They are more constant in size, and are generally a little shorter than the tubercle bacillus.

There is no evidence that spores can be seen in their interior, and they are non motile.

They stain by the Koch-Ehrlich and Ziehl-Neelsen methods, and also by Gram.

They resist decolorising, but not quite to the same extent as the tubercle bacillus. (Woodhead - Muir & Ritchie). They take up the basic aniline dyes more readily than Koch's bacillus, and can be more readily stained by watery solutions of these dyes.

Macé[3] states that they resist acids better than the tubricle bacilli, and quotes Babés' assertion, that "l'acide azotique au tiers" does not decolourise them after an hour, whilst at the end of this time, the tubricle bacilli are always decolourised. This statement is disproved by my experiments on the staining reaction of the bacillus of tubricle.

Lehmann and Neumann[2] find that the leprosy bacillus cannot be certainly differentiated from the tubricle bacillus, although it is said that the lepra bacillus is so well stained in six or seven minutes with an aqueous solution of fuchsin, that good preparations are obtained after washing in water - whilst the tubricle bacillus is not. On the contrary alkaline methylene blue is said to stain the tubricle quicker than the leprosy bacillus.

"Still all authors are now agreed that the staining reaction cannot help much in the differential diagnosis, no more than the form of the bacilli, from which it follows, that the separation of leprous and tubrculous affections in the cadaver appears often impossible"

According to Hansen and Looft tubrculosis is responsible for 40% of deaths in lepers,

(3) loc. cit. page 558.

(2) loc. cit. page 422.

and obviously this further increases the difficulty of distinguishing them.

Lehmann and Neumann give the following differential table from an article by Spiegel[23] from Unna's laboratory.

	Leprosy	Tuberculosis.
Number of Bacilli.	Exceedingly abundant in all organs and excretions	very less numerous.
Arrangement of Bacilli	In heaps like a cigar in form.	More as individuals or in irregular bunches.
Form.	Rod shaped, straight and plump.	Already curved and fine
Angles.	Sharp.	Roundish.
Granules.	Coarse	Fine
Arrangement of Granules.	Widely separated	Close together.

"These differences are naturally never so typical as here appears"

Muir and Ritchie[24] say that the presence of large numbers of bacilli situated within the cells, and giving the staining reaction of leprosy bacilli is conclusive, and consider that in most cases there is really no difficulty in distinguishing the two organisms.

[24] loc. cit. 259

Macé ③ states that the distinction from tubercle bacillus is easily made, in that the bacillus of leprosy is coloured in a few minutes by the ordinary solutions, and by Gram- whilst the former does not stain, or only after a long action of the stain.

③ loc. cit. page 564

Neisser uses the Weigert's nuclear stain as a differential means of staining.

Hansen could not obtain a pure culture of the lepra bacillus, but in 1884 Bordoni-Uffreduzzi obtained a culture on glycerine serum medium from the marrow of a leper, but was unable to preserve it.

Babès and Czaplewski succeeded later in obtaining a culture from the organs of a leper. The growth on the glycerine media, glycerine agar, glycerine serum agar and glycerine potato, was found by all observers to be delicate and slow, morphologically and biologically they are very much like the tubercle bacillus.

The growth obtained by the two last named investigators differs, according to Möller, from the true cause of leprosy, by their uncertain acid resisting power, and are classed by Czaplewski as intermediate

between the diphtheria and tubercle groups of organisms - Inoculation however failed to produce leprous changes.

E. van Houtum[26] (a Boer prisoner) has this month announced, that he has succeeded in cultivating the Bacillus of leprosy. He used a medium composed of 1/3 beef broth and 2/3 fish broth. In this the organisms grew readily and at the end of 24 hours at 36°C there is a general turbidity of the broth.

[26] loc. cit. pag. 260.

The Smegma Bacillus.

In 1885 Tavel and Alvarez[26] whilst investigating the so called bacillus of syphilis, discovered in 1884 by Lustgarten, found in the normal preputial smegma, an acid resisting bacillus - the bacillus of Smegma.

This organism is found especially in the normal smegma of the prepuce, in the secretions of the outer skin, particularly where a collection of epithelium occurs, as in the anal and genital regions, between the toes, in the folds of the groin, and below the breasts.

The smegma bacillus closely resembles the bacillus of syphilis of Lustgarten in its general morphology and staining reaction, and the fact that the latter has not been found constantly or in sufficient numbers in syphilitic tissues, makes it very improbable that Lustgarten's bacillus is the cause of syphilis. Some authorities consider that Lustgarten's bacillus is merely the smegma bacillus which has penetrated the tissues, whilst Lehmann and Neumann state that it is the general opinion, that Lustgarten's positive findings in gummas, were to be explained by a mixed infection with tuberculosis.

I may mention that I made several attempts to find Lustgarten's bacillus in the secretions of, and scrapings from, the hard chancre, whilst trying to find a means of distinguishing bacteriologically the bacillus of soft sore (Ducrey) from that of hard chancre, but without success.

I stained films according to Lustgarten's (27) method - decolourised with a solution of permanganate of potassium (1½%) and with sulphurous acid. (Lustgarten (Die Syphilis Bacillen. Wien 1886)

The bacillus of smegma as described by Tavel and Alvarez is extremely like the bacillus of tubercle in size, shape and staining reactions. It can be stained by the same method, but is said to be less resistant to alcohol. My experiments on its acid and alcohol resistance - which will be described in detail later - are totally opposed to this the generally accepted idea.

The discoverer found that inoculation experiments on animals gave negative results.

Matterstock[28] at about the same time 1885, found acid fast bacilli in smegma, which corresponded in morphologic characteristics and staining reactions, with those described by Tavel and Alvarez, but he was also unable to obtain a pure culture.

Laser[29] and Czaplewski[30] in 1897, independently obtained cultures of micro-organisms resisting decolourisation by acids, resembling the diphtheria bacillus, which they stated were identical with the smegma bacilli

Laser obtained his culture from syphilitic disease, Czaplewski from gonorrheal pus.

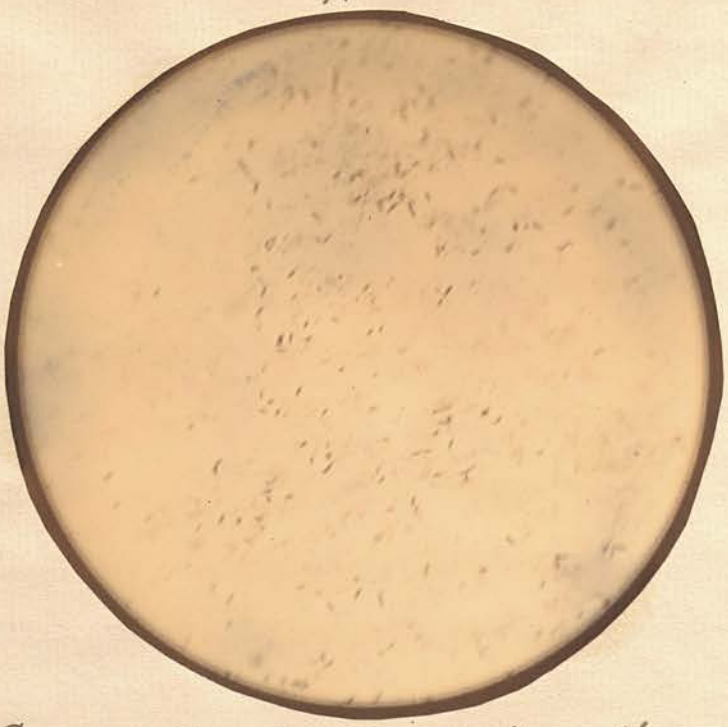
Rankel[31] denied that these diphtheria like bacilli were identical with those described

by Tavel, Alvarez and Matteistock, and he only considers those organisms smegma, which resembled the tubercle bacilli. He found that Lasers and Czaplewski's organisms did not resemble the bacillus of tubercle, that they were more like pseudo-diphtheria bacilli, and that in later generations, they lost their acid resisting power.

Möller,[17] after examining these cultures agrees with Fränkel, and states that he was unable to get pathogenic effects in guinea pigs with the diphtheria like bacilli cultivated from smegma, or with secretions containing the real smegma bacillus in abundance. loc cit p. 279

Neufeld[32] has also cultivated from smegma these acid resisting diphtheria-like bacilli, which were indistinguishable from Czaplewski's, and which possessed a moderate acid resisting power. He had repeatedly noticed an acid resisting bacillus very like the tubercle bacillus in smegma, and these he found were much more acid resisting than the diphtheria like forms. In two cases he found a great preponderance of tubercle like acid resisting bacilli over the

Scale of Magnification in all the Microphotographs
Each division = 1/40 millimetre.



Smegma Bacilli - pure Cultures: Microphotograph
Stained by Ziehl Nielsen's Method.

Smegma Bacilli in Pure Culture. Microphotograph
Stained by Ziehl Nielsen's method

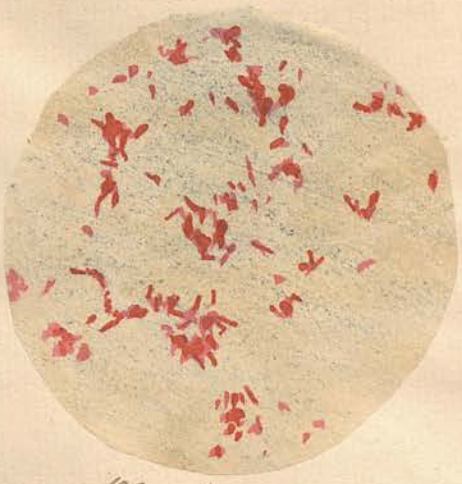
non-acid fast forms, and succeeded by cultivations in obtaining a great increase in the tubricle like forms, which he particularly remarked, possess a considerable degree of alcohol, as well as acid resistance. but he was unable to procure a pure culture of the smegma bacillus. Neufeld therefore came to the conclusion that in smegma, two types of acid fast bacilli were present, those like the diphtheria, and those like the tubercle bacillus.

I have certainly found that the bacilli in smegma vary considerably in their acid resisting power - some are almost immediately decolourised, others withstand ~~for~~ a prolonged immersion in 25% sulphuric acid - (See experimental work later).

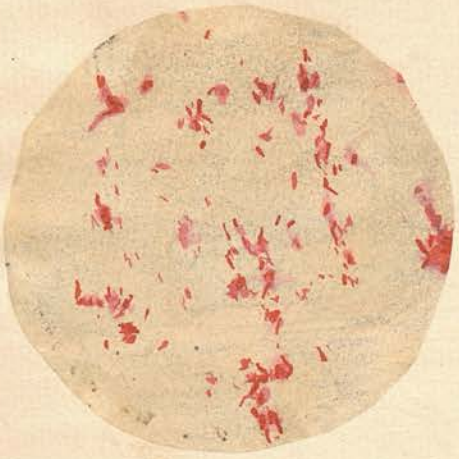
Moëller[14] has quite recently succeeded in obtaining a culture of what may be regarded as true acid fast smegma bacilli. He found accidentally whilst working on Koch's agglutination method, that in the serum of a blister produced on a healthy person by empl. cantharides, tubricle like acid fast bacilli among the epithelial cells. These were present in very

[14] loc. cit. page 280

Smegma Bacilli - pure cultures.



Old forms x 800



Young forms. x 800

from Möller modified
Centr. f. Bakt. XXXI. No. 7. 282.

Smegma Bacilli in Smegma.
(from Macé)

small numbers, but by placing the serum with fragments of skin in the incubator, he found that they were greatly increased in number after 48 hours. After 3 to 4 days, the skin floating on the surface contained a very large number of these bacilli, and by means of streak cultures on glycerine agar, he was able to isolate them.

In this way he found that human serum is the best cultivating medium for the bacillus of smegma.

Morphologically the bacilli show great variations. In young cultures they appear as slender sometimes slightly bent rods, and are deliously like the tubercle bacillus. In older cultures, they are plumper.

Culture media have great influence on their polymorphism. Especially in milk cultures - as is the case with the tubercle bacillus - alterations in form are seen, eg threads, rods with unstained vacuoles, with club like swellings, with deeply stained granules and coccothrix forms.

The bacilli show no movement.

Their staining reactions are very like tubercle. They are absolutely acid and alcohol

fast, independently of the medium on which they are grown. They are not decolourised by exposure to 3% HCl alcohol for 12 minutes, and the bacilli are stained in the cold by dilute carbol-fuchsin.

Pure cultures according to Möller[17] react to the differential stains of Bunge and Fraenkel, and Pappenheim, exactly as the tubercle bacillus. This statement as will be seen later I can entirely endorse.

[17] loc. cit. page 281

Their acid and alcohol resistance is not diminished in later generations: thus Möller found their reaction the same in the 25th generation, as in the original culture.

The smegma bacilli grow luxuriantly when air is allowed access to them, but in stab cultures there is only a slight growth along the stab. In the first generation they grow rather slowly at the temperature of the incubator. After about 3 days, the original culture appears as a clear layer of colonies. After repeated transference the bacilli get used to their artificial media, and growth takes place more profusely. After 24 hours at incubator temperature, colonies are visible, at the temperature of the

room its growth is slower. They grow on all the usual media.

On Glycerine-agar when kept at 37°C colonies appear as small dull grey ish-white scales, rounded at the edges, later these scales overlap, and appear velvety and shiny. When grown at room temperature, the dry growth persists. The water of condensation remains clear, but on the surface a slight film is formed, which creeps up the side of the glass.

On Potato grey-white colonies are seen.

In Milk they grow rapidly and luxuriantly, and this forms a good medium for growth.

The milk is not coagulated, and there is no coloured growth at the edges of the surface, as is seen in the other acid fast bacteria.

In Bouillon, the fluid remains clear, and on the surface a dry white film which runs up the side of the glass forms in 3 to 4 days. If the tube be shaken tiny fragments fall to the bottom.

Inoculations experiments are negative.

Möller[17] inoculated the smegma bacilli contained in various secretions of the skin into rabbits, hens, dogs and guinea pigs, and has never obtained any pathogenic results.

[17] loc. cit. page 282

Johannes Barannikow[33] however, writing in March 1902, states that he has obtained very different results, and from his experiments he draws the following conclusions:-

(33) loc cit page 284.

1. Inoculations of preputial smegma from a non-tubercular adult dead body, and from a healthy living child, produced in guinea-pigs local and general appearances of disease, just like that produced by inoculation of sputum containing tubercle bacilli

2. That smegma from various domestic animals (preputial and mammary) gave the same results on inoculation, viz exclusively general tuberculosis.

Both these statements Barannikow says are in direct contra-distinction to the views and investigations of Möller.

3. "It is desirable that those who assume that the so-called tubercle bacillus is the specific cause of disease, only because the acid proof organism is found in tubercle, should prove that this microbe is not the so-called smegma bacillus, and that it has not naturally or artificially been changed into one."

IV. It is necessary to investigate the entire life history of the organism.

V. That the acid-proof bacteria described by various investigators, are only developmental phases of other more highly organised microbs, and the classification into different species and genera, is based on ignorance of their complete life history.

VI. That the so-called tubercle-lepra-smegma organism, are not bacilli, but rod like developmental conditions of higher organisms.

VII. The acid fastness: and non-resistance to acids are only transitory conditions of the microbs.

So far these views have not been confirmed by other investigators.

Timothy Grass Bacillus. (Möller)

Möller [34] found this bacillus in grasses used for fodder, and as they were first discovered in the Timothy grass-phleum pratense - he named them Timothy bacilli.

I found it was not easy to discover

these bacilli on ordinary Timothy grass, and for a considerable time absolutely failed.

I made infusions of both green and dry grass, and after 12-24 hours, I obtained a fairly pure culture of the hay bacillus.

Möller in a private communication, kindly brought to my notice Lubarsch's® experiments. q. loc. cit page 196.

He obtained his grass from two different places Gehlsdorf and Barnsdorf - and placing it in an Erlenmeyer's flask, with sterilized water, kept it at a temperature of 37°C.

At the expiration of 18 hours, he found mixed with the hay bacilli, numerous acid and alcohol fast bacilli, which were according to Lubarsch easily distinguished from the tubercle bacilli, by their smaller thickness and length.

I found that mere infusions of the inflorescent part of the grass gave usually negative results, but when the whole of the grass was cut into small pieces, and infused for 12 to 24 hours @ 37°C a few acid fast bacilli, answering to Möller's and Lubarsch's description, were found. I am not at all sure that all Timothy grass contains them, but have certainly found them in other

grasses notably the alopecurus pratense, bromus erectus, and the common poa.

I found as Lubarsch state that after 48 hours, the acid fast bacilli almost completely outgrown by the hay bacilli.

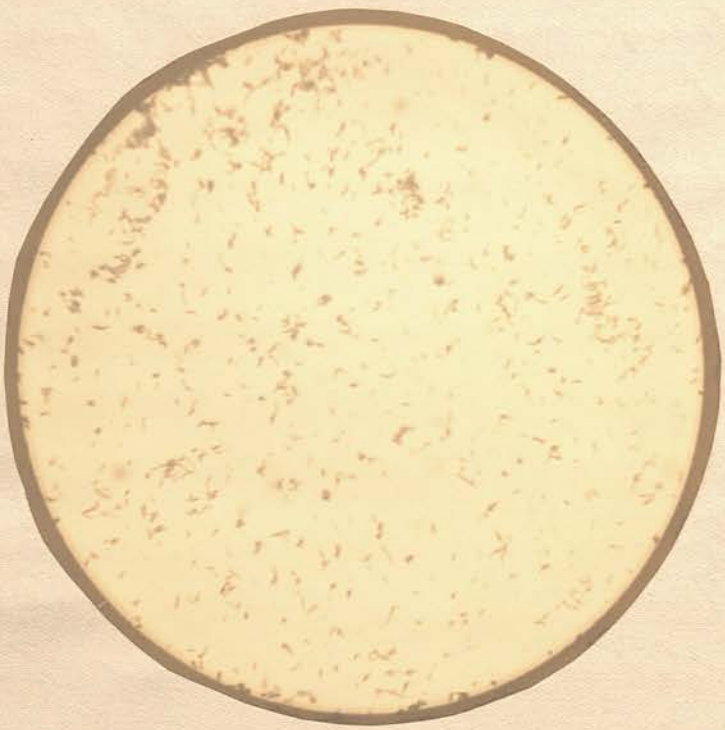
Lubarsch managed to get a pure culture by making his grass infusion with very little water, and examining hourly till such time when the Timothy bacilli were very numerous, (in one case this was at the end of 13 hours and in another 19 hours) and then by means of agar plates, isolated the bacilli.

Möller in his private communication, states that he has also obtained the bacilli from pollen.

The Timothy bacillus or grass bacillus I. takes the form of little rods, which are microscopically very like, and according to Möller often indistinguishable from, the tubercle bacilli. Lubarsch says that they are thicker and longer.

Like the human tubercle B, this bacillus often contains deeply stained granules, and also in some cases, oval unstained patches.

It divides into branches and occasionally



Timothy Grass Bacilli. Microphotograph from pure Agar Culture.
Stained by Zeihl-Nielsen's Method.

Scale of Magnification:-
Each division = 1/40 millimetre = 25μ.

club-shaped swellings are formed at one end.

True branching is seldom seen and then only in dilute bouillon cultivations, and on Länkels albumin free medium. (Lubarsch). They are not motile.

The bacillus grows on all the usual media best at incubation temperature, indifferently at room temperature.
When grown at 37°C distinct patches of colonies are seen, and sooner or later these become coloured.

On Glycerine-agar plate the colonies are after a few days of an orange-red colour, and have a moist lustre, and although at first transparent, later they become darker and more opaque.

Streak cultures on glycerin agar are of bright orange red colour, moist at first, but after a time becoming wrinkled.

In Bouillon there is a variable condition. Sometimes a thin pellicle is formed over the surface, and the fluid may remain clear, or it may become turbid, but frequently a precipitate of a yellowish colour occurs.

It grows well on potato - the colonies

appearing as yellowish moist- elevations.

Möller[36] says that the cultures differ considerably from those of the tubercle bacillus, but if the timothy bacillus is passed several times through the bodies of animals, and then grown at 37°C, it more closely resembles the tubercle bacillus, and like it becomes slower in its growth. [36] loc. cit pays 518

This pseudo-tubercle bacillus is alcohol and acid fast, and behaves in the same way as the tubercle does with the ordinary staining methods. (Moller). My experiments do not carry out this statement.

Labarsch[@] thinks that the timothy bacillus is not quite as resistant against decolourising methods, as the tubercle bacillus - and I can confirm this - and in this way its resisting power is like the bacillus of leprosy. [@] loc. cit 198.

There is he says a slight tendency for the methylene blue to mask the red of the fuchsin, but this difference is very insignificant.

When a pure culture of timothy bacillus mixed with sterilised butter is injected into guinea-pigs, generally peritonitis with adhesions form, and in

the organs are changes, which are micro- and macro-scopically very like true tubercular lesions. If it is injected into the veins or arteries of an animal, a condition - the occurrence of giant cells, epithelial cells and caseation - very much like genuine tuberculosis is produced.

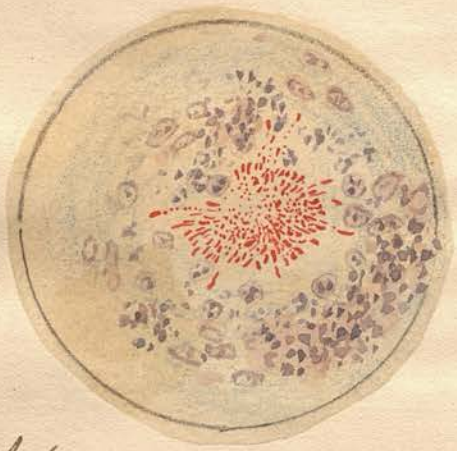
Labarsch[36] says "There can be no doubt whatever, that it is quite impossible to distinguish for certainty by histological or micro-parasitic examination, between timothy fungus tubercle, and true tubercle, the distinction can only be brought about by cultures. In all animals injected with timothy bacilli, a negative reaction to tubercalin was obtained (Quoted by Möller.)

[36]. loc. cit. page 578.

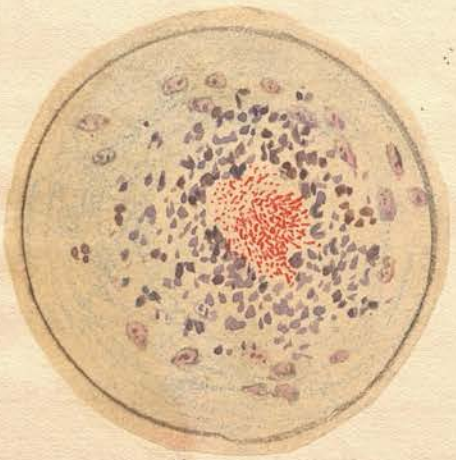
Labarsch's[37] animal experiments were briefly as follows:-

[37]. loc. cit. p. 198.

An 8 days old glycerine agar culture of timothy bacilli was injected into the kidney of a guinea-pig. At the end of 13 days a small piece of kidney at the point of injection was excised, and a small yellowish mass the size of a lentil found, apparently of caseous nature. This microscopically showed typical tubercular appearance.



Actinomyces development of Moëllers Grass Bacillus II. Section through the kidney of a rabbit 14 days after inoculation.



Actinomyces development of Möllers Timothy Bacillus Section through nodule in kidney of rabbit 30 days after intra-venous inoculation.

From Abbott and Gildersleeve.
Centr. f. Bakt. XXXI. No 12. page 550.

viz. a mass of large epithelioid cells, among which were Langhan's giant cells, and around these a proliferation of uninucleated round cells. In the centre of this nodule, the bacilli were arranged in typical radial manner like actinomyces. The clubs of these were not stained with Ziehl-Neelsen's solution, but appeared colourless or took on the blue of the methylene blue - just as the clubs of the true tubercle bacilli do.

On the 31st day after the injection, a piece of a nodule which had formed on the kidney was excised, and it was seen that caseation had increased, and the genuine Langhans giant cells were more numerous. The radially arranged masses of the bacilli were still present, and although not more numerous than on the 13th day. they were larger, and the clubs longer and thicker.

Whether the timothy grass is pathogenic for man is not known. Lubarsch however inoculated himself on the forearm, and on the 8th-10th day a small hard swelling developed. This was found to be inflammatory changes around sweat glands. Rods but not actinomycotic forms of timothy bacilli were present.

Grass Bacillus II. (Möller)

Möller[37] described this as a new acid fast alcohol fast Bacillus, of the tubercle bacillus group, which shows genuine branching

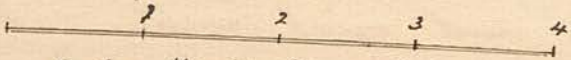
37. loc cit. page 369.

He found it in the pollen, dust on the stable floor, in fodder and grasses generally, and was able to isolate it on glycerine plates.

It takes the form of little rods, sometimes like cocci, in fluid medium, and morphologically, and in its reaction to stains it is very similar to the tubercle bacillus, but is thicker. Sabarsch says, that when grown upon meat media, it appears as little rods, which are longer and thicker than the tubercle bacillus.

Like the other pseudo-tubercle bacilli, viz the timothy grass and mist bacillus - it is absolutely acid and alcohol fast, especially in young fresh cultivations. In young fluid cultures, threads and branching forms are seldom seen, but in older cultures, especially those made on solid media, after 4 or 5 days these are frequently found, and they obtain a pale red with Ziehl-Neelsen stain, having lost somewhat the power of resisting acids.

Grass II Bacillus - Microphotograph taken from pure Agar Cultures.
Stained by Ziehl Neelsen's Method.



Scale of Magnification each division = 1/40 millimetre
Each division = 25μ

On agar the growth is luxuriant; and when kept at 37° C on glycerine agar-streak, small delicate drop like colonies form after two days, and these later run into one another.

The culture is then somewhat glistening, and for the first few days colourless, but afterwards it takes on a yellowish tinge. In the water of condensation, which remains clear, little skin like pellicles form, which eventually sink to the bottom.

Lubarsch[9] in his account says, that unlike the radial fungi generally, its growth on agar is soft and pulpy.

On Potato cultures kept at 37°, the growth comes up luxuriously, and forms thick grey white colonies.

In Milk the growth is very rapid, and is acid in reaction after two or three days.

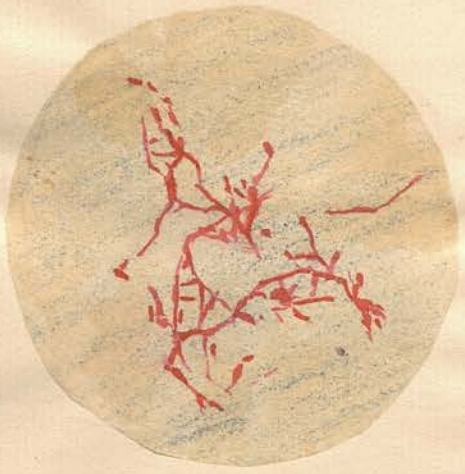
In Bouillon. after 3 or 4 days, at the temperature of the room no turbidity occurs, but by shaking the tube a thread like sediment falls.

On the surface, a whitish-grey skin forms, which tends to grow up the side of the glass.

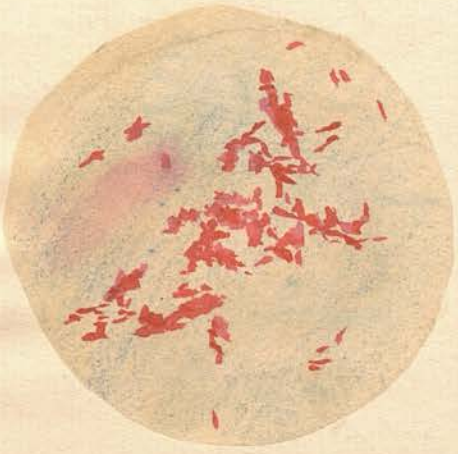
The bouillon however remains clear.

Along the streak on Gelatine, after 4 or 5 days at 20° C a thick greyish white colony forms -

Grass Bacillus II.



Pure culture 3 days on Glyc. Agar.
showing true branching.



Pure culture –
rod forms.

Modified from Mösller
Cent. f. Bakt. XXV. No 11. p. 373.

The stab in ~~glycerine~~ gelatine stab cultures is well covered - but there is no liquefaction.

Möller[37] has stained these organisms by most of the tubercle stains, viz Fränkel's, Ehrlich's, Czaplewski's, Gabbet's and Ziehl Neelsen's methods - and finds that the bacilli in their young state resist decolouration by mineral acids and alcohol, just as the tubercle bacillus. They are stained by Gram's method. They possess amoeboid movement only in their young condition.

(37) loc. cit. p. 370

In size the bacilli vary considerably. Most of them are about 1-5 μ long and 0.2 - 0.4 μ broad. Especially long forms are found in the nodules which occur in infected guinea-pigs. They generally have a slightly bent shape. Long branched and unbranched thread forms, are found at the margins of the colonies, especially those grown on glycerine-agar for 3 or 4 days at 37°, and sometimes fragment- and cocci-forms occur, particularly in milk cultures.

The threads often have deeply stained granules, which are much broader than the body of the organism. Swellings at one or both ends of the bacilli are sometimes seen

The branched forms are usually made up of a long thread, from which other thread like branches, or short club like curlings start off at right angles, unlike the acute angled branching of Cladothrix. The fine branches often divide again.

Möller states that he has seen similar branching of the tubercle bacillus in sputum and Kral and Dabard have found the same in the tubercle bacilli of cold blooded animals.

Topf of Halle considers with Möller that these bacilli, like some form of tubercle bacilli, show true branching, and not the false branching of the cladothrix, which would tend to show that these like the tubercle, are not true bacilli, but forms more allied to the actinomyces. This opinion seems to be steadily gaining acceptance.

Lubarsch incidentally mentions that the grass bacilli II, as found in cultivations, are rather less resistant to acids and alcohol, than the tubercle bacilli, or some other of the pseudo-tubercle bacilli, and this is more decided in preparations taken from the bodies of

infected animals.

As a result of my experiments, I came to the conclusion that they are decolourised, the earliest of all the pseudo-forms which I examined.

Inoculations into animals. According to Möller [37], guinea pigs intraperitoneally injected with a pure culture of grass II bacilli, died at the end of 4 to 6 weeks.

When milk cultures were similarly injected, the animals usually died in 10 to 20 days, and presented very much the same microscopical appearances, as the infected areas in animals injected with Koch's tubercle bacilli. Whilst in pure tuberculosis, tubercle bacilli are found very sparsely in the caseated masses, in the nodules caused by the injection of these pseudo-tubercular bacilli. Enormous masses of acid fast bacilli were found in caseous masses.

Histologically the grass II, as also the grass I or timothy bacilli, give rise to tubercular-like processes.

Lubarsch [9] as a result of experimental inoculation of cultures of pure grass II

[9] loc. cit. 209

bacilli into guinea pigs, found that the radial actinomycotic arrangement of the bacilli, though present, was not nearly as frequent as is the case with inoculation of timothy and mist bacilli.

He concludes that the grass II bacillus is not quite as pathogenic for guinea pigs as the other two varieties, whilst Möller thinks, that they possess greater virulence for guinea-pigs, especially when milk cultures of the bacilli are given intraperitoneally.

The latter remarks, that the growth of the grass bacillus II on agar at 20°C, after its passage through the bodies of animals, looks almost identical with that of the tubercle bacillus, which he obtained and cultivated at 20°C from the blind worm.

According to Möller[37] the grass I Bacillus is easily distinguished from all acid and alcohol fast bacilli of this group, by the occasional occurrence of true branching.

He has found, that by passing the timothy bacillus through animals, and keeping them at 37°C on glycerine agar, ~~that they~~ closely resemble the true tubercle bacilli.

They all - the timothy grass I, the grass II

and mist bacilli, produce in guinea pigs a miliary tubercular disease, and all show thread forms, club forms, oval unstained vacuoles (the so called spores) and dark granules in the bodies of the bacilli, and like the genuine tubercle bacillus, are acid and alcohol proof.

The grass ĩ bacillus shows the same branching that is sometimes known to occur in the tubercle bacillus, and it would seem probable according to Möller, that rod form of the bacillus of tubercle is only a phase in the cycle of development of a more highly organised fungus, which like grass ĩ bacillus, may exist independently as a saprophyte. Möller like Czaplewski, Ransome and ~~Koch~~ others has managed to grow the tubercle bacillus at the ordinary room temperature.

Möller has found these forms of pseudo tubercle bacilli widely distributed in the vegetable world, and questions whether their life is symbiotic - quite harmless to the plant, or whether they exist as a cause of plant disease, and which under certain conditions might affect human beings and animals.

The Mist Bacillus or Manure Bacillus (Möller).

In the fresh excreta of cows, asses and other herbivora, as well as in a manure heap some months old, Möller[16] found another acid and alcohol resisting bacillus, which he has called the Mist or Dung bacillus.

It closely resembles the smegma bacillus in its structural appearance, and in its reaction to stains, and in cultural and pathogenic characters is very similar to the grass ii bacillus.

I had no difficulty in at once finding this organism in fresh and old cow-dung, and in the green slime at the bottom of an old rain water tub, I discovered it in enormous numbers. In cow dung it generally occurs in small numbers, and although mixed with many other substances - especially the spores of fungi - which also resist decolourisation by Ziehl-Neelsens method, yet it is so definite a bacillus that it could not easily be overlooked, particularly in films which have been subjected to the decolorising agent (acid), for some time.

I found that this bacillus can be more easily distinguished from the tubercle, and

other of these acid fast organisms, by the following points -

i That it is more constant in size and shape,

ii That it is shorter - and plumper, and generally appears more like a grain of wheat pointed at both extremities

iii That it stains very deeply by the fuchsin but shows less internal structure - and

iv. That it is probably - next to the genuine tubricle bacillus the most resistant against the decolorising effects of acids etc.

Lubarsch[1] whilst recognising its close resemblance to the timothy bacillus is able to draw certain points of distinction between them it, and the other closely allied species.

[1] loc cit page 209

On agar both organisms grow in a similar manner.

Bouillon never becomes diffusely turbid by the dung bacillus, as is usually the case with the timothy fungus -, and colonies are rarely found at the bottom of the tube, which is generally the case with the latter.

On Gasperini's medium, the timothy bacillus grows luxuriantly - the mist bacillus sparingly. The mist bacillus, less frequently forms true

branching -

Further slight differences be also found in the effects on guinea pigs. When injected into the kidney of these animals, the results were not quite as like true tubercle, as the timothy bacillus produces, as in the nodules the large uninucleated elements like epithelioid cells were not so frequently found, but true radial arrangement of the bacilli is much earlier. In one case typical radial masses with clubs were found as early as the 6th day.

In order to determine more fully the occurrence of these radial or actinomycotic forms of the mist bacillus in animals, Lubarsch injected a culture of the dung bacillus directly into the kidney of a guinea pig.

In one case at the end of 6 days, and in another on the 8th day, radial masses of bacilli with clubs were found at the point of inoculation, lying in the giant cells, and partially surrounded by leucocytes. These differed from those resulting from the injection of timothy bacilli - in that after staining with Birch-Hirschfeld's method - the threads are only faintly stained generally a brown colour, and part of the clubs a violet colour.

The Butter Bacillus. (Petri - Rabinowitsch)

Owing to the fact that so-called tubercle bacilli were found in such alarming frequency in butter and milk, investigations were made independently by Petri[38] and Rabinowitsch[39] to determine whether these organisms were the genuine tubercle bacilli.

Both these observers found in butter and milk a bacillus, which although it is acid and alcohol fast, is not the true bacillus of tubercle. As the organisms isolated by these investigators are so much alike, they are generally described as the Petri-Rabinowitsch bacillus.

This organism takes the form of little rods, very much like the tubercle bacillus, but somewhat thicker. According to Lubarsch, it is generally longer and thinner than the timothy and mist bacillus.

Deeply coloured granules are found in the body of the bacillus, as in Koch's bacillus.

It resists decolourisation by alcohol and acids, exactly as the tubercle bacillus, but in cultures according to Möller, it is not as resistant to acids, as the last named bacillus.

It grows well at the room temperature, and at incubation temperature there are visible signs of growth after 24 hours.

When grown on the ordinary nutritive media, the colonies distinctly differ from those of tubercle, but closely resemble the latter on Proskauer's albumin free medium, and in bouillon.

According to Lehmann and Neumann② when grown on glycerine-agar plates, the growth has the appearance of wrinkled, irregularly dentate scales, of a transparent greyish white colour. Later they become opaque, of a brown-grey colour, and are irregularly marked.

② loc. cit page 431.

Streak cultures on glycerine agar, appear as wrinkled dry formations, very like that of true tubercle, and easily mistaken for it in the young condition. Later the growth becomes of a somewhat orange to copper red colour. (Rabinowitsch)

In bouillon, a thick wrinkled pellicle is soon formed, but the fluid remains clear with scarcely any precipitation. It has a disagreeable ammoniacal odour.

Grown on potatoe, the colonies are at first white to orange colour, but soon become wrinkled, and afterwards very like that seen on glycerine-agar. The growth is dull, dry, and

shows little or no glistening appearance.

Lubarsch® emphatically contradicts Lydia Rabinowitsch's statement, that the butter bacillus is identical with the timothy and dung bacillus of Möller. He states that the organisms differ in morphology, in culture, and in the results of animal experiments. ® loc cit. 209.

The butter bacillus is longer and thinner, and especially when grown in thin bouillon shows genuine branching, and clubs more elegantly, than the other pseudo-tubercle bacilli.

On agar, he says the butter bacillus remains colourless much longer, and does not become a yellow colour till the 8th day, and then does not possess the intense colour that the colonies of the timothy and dung bacilli do.

Unlike the timothy bacillus, a wrinkled pellicle is formed on the surface of bouillon after 48 hours, and a precipitate soon occurs leaving the bouillon translucent, whilst early clouding of bouillon is characteristic of the timothy fungus.

Animal experiments produced less change than Mollers bacilli. When injected with butter into guinea pigs, Möller found changes which macro- and microscopically might easily be mistaken for genuine tubercle. When injected in pure culture

alone, less effects were produced than when butter was injected with it.

Labarsch found in the injected areas, resulting from the injection of butter bacilli, the radial arrangement of the bacilli with club formations.

Langhan's giant cells, nests of epithelioid cells, and typical tuberculous caseation, according to Rabinowitsch, are never found in the foci of the disease.

Korn's butter-bacillus.

Korn (40) has isolated another acid fast bacillus from butter, which he has termed 'Bacillus suburgensis' which he claims differs from the Petri-Rabinowitsch's butter bacillus morphologically, culturally, but especially in its action on animals.

When grown on glycerine-agar the colonies are of a white colour, later they become wrinkled and coppery red.

"Korn gave the following as characteristic of his butter bacillus.

1. Stains by Ziehl-Neelsen's method especially well, and is little influenced by acids.
2. Uniform uninterrupted growth in gelatine stabs.
3. The surface of the agar culture is depressed.

in the centre the peripheral zone being elevated.
4. Upon bouillon it produces a disagreable but not ammoniacal odour. These characteristics are partly inconstant, partly unessential.

Inoculation with large quantities of pure cultures, and diseased organs, caused no disease in guinea-pigs, rabbits, chicken and pigeons.

White mice are readily infected by the intra-peritoneal injection of 0.6cc of a suspension.

The animal dies in from 4 to 40 days, and presents massive nodules in all the abdominal organs" (Lehmann and Neumann.)[2]

[2] loc cit 432.

All bacteria resistant to acids, cultivated from milk and its derivatives, show a great resemblance to the grass bacillus. Taking into consideration the habitat of the latter namely cattle fodder, we are certainly justified in regarding the milk and butter bacilli, as varieties of the grass bacillus. Such differences as there are, and these are very slight, may be explained by the passage through the bodies of animals. (Möller[33] at Brit. Congress Tuberculosis) loc cit page 489.

Acid-Resisting Bacilli found in non-tubercular Secretions and excretions of Man.

Since attention has been drawn to the fact that acid-fast bacilli may occur much more generally than was supposed, evidence has been accumulating regarding the presence of these harmless pseudo-tubercle bacilli, in various secretions and excretions of man.

Fraenkel stated in 1898, that he had repeatedly found acid resisting bacilli in the sputum of a case of non-tubercular gangrene of the lung.

Pappenheim also discovered what he took to be tubercle bacilli - in that they were acid-fast bacilli - in the sputum of a case which post-mortem examination proved was not tubercular.

Lydia Rabinowitsch also found similar organisms under like conditions, and proved by pure culture that they were probably a variety of the Koch bacillus.

Marzinowski found acid fast bacilli in the crypts of the tonsil, Karlinski in the healthy and diseased nasal cavity.

Mironescu cultivated an acid resisting bacillus from the fæces of a case of supposed typhoid.

Möller [16] has he says, frequently found pseudo-tubercle bacilli, in the mucus from the nose and pharynx, coatings on the tongue, sordes on the teeth, and eruptions on the tonsils.

[16] loc. cit page 6

During an attack of bronchitis, he found in his own expectoration, small purpid nodules which contained acid fast bacilli in great numbers. By further investigation he proved that these were not genuine tubercle bacilli, and this was corroborated by the fact that his lungs remained absolutely healthy after three years.

He further mentions, that in the fluid obtained from a case of acute pleurisy, he found acid fast bacilli, which could be mistaken for tubercle bacilli. He placed some of this fluid mixed with cultivating medium, in the incubator and after a few days noticed a considerable increase in the acid resisting organisms. By reinoculating, he found that these grew at the temperature of the room, a clear proof that they were not real- but pseudo-tubercle bacilli. The after history

of the case proved that it was not one of tubercular disease.

Lubarsch[41] in drawing attention to the prevalence of acid-fast organisms resembling the tubercle bacillus, and points out the unreliability of the microscopic examination alone, as a means of diagnosis. He mentions the following illustrative cases.

i. Case in which rods stained well with Ziehl-Neelsen's method were found in sputum and phthisis diagnosed. As the organisms were unusually short and thick - injections were made into the peritoneum of guinea-pigs. No tuberculosis developed, and the further history of the case negatived the diagnosis of phthisis.

ii. Case of carcinoma of the stomach, with pleural and pulmonary metastases, and purulent bronchitis. The sputum contained acid resisting bacilli which were innocuous to guinea pigs. On post mortem exam^n no signs of pulmonary tuberculosis were found. The bronchial secretion (P.M.) also contained these bacilli.

iii. Secretions from a bronchial cavity contain-ed pseudo tubercle-bacilli - which produced

negative results when injected into guinea pigs. No evidence was found at a Post-mortem examn of tuberculosis.

IV. In an abscess near the hip joint, numerous acid resisting bacilli were found, some of which were morphologically identical with the tubercle bacillus, others were shorter and thicker. Attempts to cultivate them, or to infect guinea pigs failed. The after history was opposed to that of tubercle.

V. In the cystic swellings on the forearm of a medical man, who had had symptoms of tuberculosis as a child. he found acid resisting organisms morphologically identical with tubercle. but cultures and injections into guinea pigs were negative.

He asserts that he proved conclusively that the 2nd 4th and 5th cases were not tuberculous, in spite of the presence of acid resisting bacilli. In the other cases the bacilli might have been attenuated or dead. Against this assumption he points out, that guinea pigs are so sensitive to tuberculosis. that they may be killed by the injection of fluids, in which no tubercle bacilli can be demonstrated microscopically.

"It follows" he says "that in doubtful

cases

which include all in which an examination of a pathological liquid is likely to be made, a bacteriological examination even if the result is positive as regards the presence of acid resisting organisms, is insufficient on which to base a diagnosis. Since cultures of the true organisms only succeed in a majority of cases, the only remaining reliable test is the injection of the fluid into susceptible animals." (Brit. Med. Journ. abst. Epitome. June. XIV. 1902).

Pathogeny and Differentiation of the Pseudo-tubercle Bacilli generally.

Möller[16] states that all the pseudo-tubercle bacilli, bacteria resistant to acids, except the bacillus smegma, have this in common, that they cause a tuberculous disease (Knotchenkrankheit) in the usual animals operated up, the true tubercle bacilli always, but the pseudo tubercle bacilli only in a limited number of cases, and under certain conditions.

[16] loc. cit. pag.

The pseudo tubercle bacilli are especially

virulent if injected with butter into animals, and when inoculated intraperitoneally, always causes peritonitis with extensive induration.

If genuine tubercle bacilli together with butter are injected intraperitoneally into animals, typical tuberculosis does not result, but indurative peritonitis, just as is the case with the pseudo-tubercle bacilli and butter.

He inoculated six calves with human tubercle bacilli, grass bacilli and pseudo-pertussis bacilli, with and without butter.

The calves before operation did not react to the tuberculin test; the butter in all cases was sterilized. As a result of these experiments, he found that the pathological action of the tubercle bacillus hominis in calves in no wise differs from that of the pseudo-tubercle bacillus; that tubercle bacillus hominis alone produces the same pertussis-like appearance in calves, that the pseudo-tubercle bacillus alone does: and that the tubercle bacillus hominis with butter, causes the same appearances of disease, as the the pseudo-tubercle bacillus with butter does.

Lubarsch© and later Mayer and

Holscher have proved by numerous experiments on guinea pigs, rabbits that the pseudo-tubercle bacilli can produce, even without the presence of butter, a diseased condition closely resembling tubercle.

The resemblance of pseudo- with real-tuberculosis is so close, that Möller says even a practised eye may easily be deceived, but on further examination considerable differences will be found.

Kruse[21] in Flugge's Bakteriologie has called attention to a method of differential diagnosis by means of inoculations. If true tubercle bacilli be injected into the anterior chamber of a guinea pig, tuberculosis of the eye always results, whilst if pseudo-tubercle bacilli are injected in the same way, no such appearance is seen.

Subcutaneous injection of true tubercle bacilli always results in a general infection, whilst pseudo tubercle bacilli do not produce such a result, an abscess only forms at the point of inoculation.

As to the differences in their transference, true tuberculosis can be caused by means

of the infected organs from one animal to another, but this cannot be done with pseudo-tuberculosis; animals can only be inoculated with a pure culture of the bacilli, taken from the infected organs.

The clinical results of the inoculation of real- and pseudo-tubercle bacilli differ. Animals inoculated with true tubercle bacilli, soon after the injection show signs more or less marked of illness - They lie about as if feeble, they are feverish, their appetite disappears, and in spite of attention as to the food etc, they become thinner and weaker and die. Post-mortem examination shows general tuberculosis.

Animals inoculated with pseudo tubercle bacilli soon after the injection show somewhat similar signs, but these quickly pass off and they run about, eat well, increase in weight and appear quite healthy - and yet one is surprised to find after killing them, distinct pathological changes.

Further differences are seen histologically. True tubercles are generally of a soft proliferative nature, a condition which later goes on to caseation - whilst pseudo-

tubercle are of a more inflammatory type, with a tendency to the formation of abscesses.

Most important however is the fact that the tubercle bacillus when inoculated, increases in number, whilst the pseudo-tubercle bacillus appears only as a foreign body, and there is no increase in its number, except when it is present in large masses, or when carried by the blood or lymph stream. When the pseudo-tubercle bacillus is only injected in very small quantities no tubercular like appearances occur, the greater the bulk of inoculating material used, the more widespread the pathological changes - whilst with pure tubercle bacilli, this is of no importance.

It is stated that 40 virulent tubercle bacilli are sufficient to produce general tuberculosis in a guinea pig

It has not been proved that the pseudo-tubercle bacillus is pathogenic for man. and judging from general observations this is probably not the case -

In no case, not even when these bacilli have been found in pathological conditions, can they be proved to have any etiological connection with any disease in man

The close resemblance between the

pseudo- and genuine tubercle bacillus in morphology, and staining reaction, is only an apparent relationship - the fundamental fact is, that Koch's tubercle bacillus, and it alone, is capable of producing true tuberculosis in man.

The timothy bacillus may be made to acquire the slow growth of the bacillus of tubercle, and in cultures they may be alike, and the tubercle bacillus may become accustomed to a more rapid growth at a lower temperature, yet they will always remain distinct organisms. They cannot be transformed the one to the other.

Most of the acid resisting bacilli show an essentially saprophytic growth; the tubercle bacillus only shows such a growth in cultures - it is only found in disease products.

An Acid-fast Streptothrix pathogenic to Man and Animals.

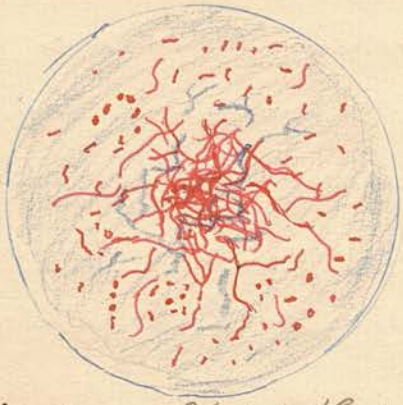
Majors Birt and Leishman (42) of the R.A.M.C recently (April 1902) report a case in which they found a streptothrix which was acid-fast

(42) loc. cit pgs. 120.

Briefly, the case was that of a Private, who, whilst beleaguered at Ladysmith contracted fever and dysentry. On his arrival at Netley, he was found to be suffering from right pleural effusion and fuat enlargement of the liver. On examining the reddish muco-purulent expectoration, acid fast bods resembling the tubercle bacilli were found, and at the same time a few segmented branching filaments, also acid-fast, were seen, and at first thought to be actinomycotic forms of the tubercle bacillus.

From the pleural cavity, and from what proved to be an abscess of the liver, a streptothrix was isolated and cultivated. Here it was seen to be more branching than in the sputum.

In scrapings from the pneumonic nodules, and in sections of the infected areas in the lung, acid fast organisms closely resembling



Acid-fast Sheptothrix of.
Burt & Leistman.
Stained with Zeil-Nielsen decolourized
with 25% H_2SO_4 and counter stained
with methylene blue.

those figured by D'Arrigo, were found.

In the pus, the streptothrix occurred as a fairly open network of long thin segmented threads, with lateral branchings at right angles.

In length, they stretched almost across the field of the microscope (1/12 oil immersion).

The width, which was fairly uniform, measured about 0.5μ, and the threads showed no signs of clubs or spore formation.

The threads stain well with all the basic aniline dyes, and retain Gram's stain.

After staining with Ziehl-Neelsen, and decolourising with 25% sulphuric acid and alcohol, they remain deeply stained.

Cultures. The growth is very slight if at all at room temperature.

On Gelatine plates, after 5 & 6 days incubation at 22°C, white circular colonies formed, which resembled balls of cotton wool - snow white by reflected light, and slightly yellowish in the centre by transmitted light. No liquefaction of the medium occurred after 3 weeks incubation.

On Agar. The growth is rapid. After 36 & 48 hours snow white dry powdery growths appear, which later become pale pink.

In nutrient Broth, after 24 & 36 hours at 37°C

Scale of Magnification

Each division = 1/40 millimetre or 25 μ.

Streptothrix - Burt v Leichman. 3 days old culture in Bouillon - Stained by Löffler - Neelsens Method.

white specks are seen on the surface. These increase in size, become more spherical in shape, and assume a pink colour above the liquid, a white colour below.

Potatos forms a good medium, and after 48 hours a copious dry white growth like a splash of plaster of Paris is seen. The pink tint develops as early as the 3rd or 4th day. Free growth takes place at 22°C, optimum growth is at 37°C.

The streptothrix appears in cultures in two forms, a fine branching of mycelial threads, and a so called strepto-coccic form. On dry media the threads break up into what the authors call arthrospores, i.e. a series of oval segments, and these constitute the white powder seen in cultures. In fluid media, the part of the colony above the liquid consists of arthrospores, that below of branching threads.

The acid fastness of these varies according to the age of the growth, as is said to be the case in tubercle B. (Klein) and in grass ψ B (Möller). When young, the threads and arthrospores retain the fuchsin intensely,

but gradually the threads lose their acid fastness, till in old cultures they may be decolourised. The arthrospores retain their resistance to acids even in old cultures. They behave in the same way with Gram's stain.

In cultures of some months old, the observer noticed that bodies deeply stained black by Weigert's method were seen in the threads but nothing like club or involution forms were found.

When inoculated intraperitoneally into guinea-pigs death occurred in from 5 to 6 weeks and lays collections of caseous matter were found in the peritoneal cavity.

When inoculated under the skin similar caseous matter formed and in both cases in this the organisms were found chiefly in the form of threads.

Major Leishman I.M.S. of Netley has forwarded to me a culture of this streptothrix, and I shall later describe the extent to which it resists acids etc. (see page 115).

The Streptothrix of Eppinger.

The streptothrix of Eppinger [43] 1890, or what he terms 'a new pathogenic Cladothrix producing pseudo-tuberculosis' was isolated from an abscess of the brain.

Eppinger from its mode of branching termed it Cladothrix asteroides.

Rossi Doria in 1891 recognising that the branching was not the false branching of a cladothrix called it 'Streptothrix Eppingeri' and Flugge also speaks of it as a Streptothrix.

Lachner-Sandoval pointed out that it cannot be classified with the oosporae, as these have tubular filaments with double contours, nor with the Streptothricae, in which segmented tubular branching hyphae bear brown spores, and he therefore calls it 'Actinomyces asteroides'.

Mac Callum [44] found this fungus in the pus from a peritoneal abscess. He describes it as a branched filamentous organism, with no evidence of septum formation. The branching is true not dichotomous.

It stains readily with ordinary aniline dyes and especially well with methylene blue, and

retains Gram's stain.

The staining is not regular, clear unstained areas or deeply stained granules are found in its course.

He found that when stained by Nisser's method, very distinct blackish-blue granules were seen in its interior. There is apparently no evidence of spores.

It is pathogenic for ordinary laboratory animals - producing widely disseminated lesions of the nature of abscesses, and in the surrounding granulation tissue giant cells are often seen.

Labarsch[1] found that by inoculating a culture of this organism into guinea-pigs that live radial growths - actinomyces like - were produced - similar to those which he found were produced by the tubercle and pseudo tubercle bacilli.

[1] loc. cit. pag. 211.

Neither MacCallum, Macé nor Labarsch mention whether it is acid-fast, or stains by Ziehl-Nielsen's method.

Berestnew[2] states that it does stain by this method, and Bat and Leishman[42] speaking of acid-fast microbes say, that the "Streptothrix bovis (Nocard) stained in this manner. The

[2] loc. cit page 450.

[42] loc. cit pag. 121.

streptothrix isolated by Eppinger presented the same characteristic"

Burt and Leishman admit that this so called streptothrix of Eppinger is not unlike the one which they have found in its morphological form, but differs from it by growing to some extent anaerobically, by the characteristic growth in bouillon, dense layers of feltwork which sink to the bottom and are renewed again and again, and by the agagy tint which the cultures assume.

Another acid fast organism the Streptothrix bovis found by Nocard[45] in 1898 in the disease known as "Farcin du Boeuf". This is apparently a rare affection of oxen, occurring in the island of Guadeloupe, characterised by suppurative inflammation of the lymphatic glands and vessels.

[45] loc. cit. pag. 529.

Nocard states that it does not stain well with the ordinary aniline dyes.

According to Berestnew it stains by Zeehl. Neelsen's method. Burt and Leishman say that Nocard found that it obtained in this way.

Lehman & Neuman[2] state that Nocard says it does not stain with Zeehl. Neelsen's method

[2] loc. cit. p. 448.

The Morphological Characters of the Tubercle and Pseudo-Tubercle Bacillus compared.

The following description is that found, by comparing on the same slide, the various pseudo-tubercle bacilli stained and decolorized in exactly the same way with Zehl Nelsen's carbol-fuchsin-decolourising for a few minutes in 25% sulphuric acid, and counter stained with weak watery solution of methylene blue.

1. Tubercle Bacilli - are stained a brilliant red colour. They are of slender but fairly uniform breadth, usually somewhat bent and often showing a somewhat braided appearance. In cultures, especially the older the internal structure, the alternation of clear and darkly stained areas are more pronounced.

2.(a) The Smegma Bacilli in Culture - are stained of a darker red, owing to their being slightly affected by the after staining with methylene blue, and are more opaque and darker. In size, they are very variable, but are generally shorter and much thicker than the tubercle bacilli. There is a tendency to the

form of a wedge or spindle rather like a diphtheria bacilli. Cocci forms are more frequent. There is much less signs of internal structure.

(3) Smegma Bacilli in Smegma. The bacilli are of very variable ~~size~~ size, but generally shorter than the typical tubrole bacilli. The majority are in the form of opaque rods with more or less rounded ends, not pointed. They do not present the appearance of the Bacillus diphtheria, quite as much as when seen in cultures. A few are like elongated cocci, some are thread like and these generally stain faintly, and others are club like. They present very little signs of internal structure, and the beaded appearance so commonly seen in the tubrole bacilli is generally absent.

Their width varies considerably but generally they are distinctly thicker, coarser and plumper than the tubrole bacillus. but very many of them cannot be distinguished from the latter. Their colour as I noticed in all the pseudo tubrole bacilli is darker, denser, less transparent than Koch's bacillus. A very few show

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a bays centrally placed deeply stained granule. like I have found in ~~the~~ a few T. bacilli in sputum.

Sometimes I have met with a few exceedingly thick forms, twice or three times the thickness of the ordinary bacilli.
The majority are somewhat thicker at one end than the other, and most are slightly bent.

3) Timothy Bacillus. are stained of a darker red. Their size and shape are very variable generally thicker and coarser than the tubercle bacillus. The sides of the bacillus are not parallel. They are thicker in the centre and tail off towards the extremities. They show very little signs of internal structure, and a dense appearance. A few short-like forms are seen in cultures which stain faintly and are not well defined. These are often the longest forms present.

4) Grass II. Bacillus. are stained an opaque dark red colour. The extremities are squarer and the sides more parallel than many of the pseudo-tubercle bacilli.
Although very variable in size, they are usually longer than the other pseudo- but distinctly

plumper than the tubercle bacilli. Short cocci forms are however often seen.

These are the only pseudo-tubercle bacilli in which I could detect fine granules, when stained by Neisser's method. (vide infra).

5) The Mist or Dung Bacillus is least like the Tubercle Bacillus of all the acid resisting bacilli. It is the shortest, and thickest. It shows no internal structure; and has usually both extremities pointed, and is much more spindle shaped. not unlike a grain of wheat. In older cultures however, it becomes longer and thinner and then more closely resembles the tubercle bacillus.

6). The Streptothrix of Bist and Leishman.

Thanks to Dr Leishman of Netley, I was enabled to study his acid-fast streptothrix in agar culture one month old - and also in agar cultures of 3 days, and in bouillon three days. Although the fungus varies according to its age - yet what struck me most was the prevalence of cocci or rod forms in the young as well as in the old cultures on solid media. The filamentous forms are best seen in liquid media - at the bottom of the tube in the form of a flocculent light precipitate.

At first sight film preparations made from a month old agar culture, have the appearance of the mist bacilli, i.e. very short thick rods, but on further examination, especially in young cultivations, the organism is seen to consist of cocci, short rods, long bacilli and a network of filamentous thread forms mixed up together. The cocci forms are not usually quite round. They show a tendency to the form of a bacillus. The short rods vary in length from that of a coccus to rods the length of the tubercle bacillus. The long rods are considerably longer than the tubercle bacillus and merge into the branching network of threads. Stages of the segmentation of the threads into a chain of rods or into streptococci forms are often seen. The so called spores are fairly uniform in size and have an oval shape. The thread forms show true branching and form a more or less dense network. I think that in old cultures at least, this organism could easily be mistaken for the mist bacillus, but here the width of the coccal-bacillary and thread forms are very constant.

I shall describe their acid resisting power later.

Determination of the Degree of Resistance of the Acid-fast Bacteria towards Acids, Alcohol, etc.

I have made the following experiments to determine to what extent the acid-fast pseudo-tubercle bacilli viz the Bacillus of Smegma, Timothy Grass, Grass II, Mist or Dung and the Streptothrix of Birt & Leishman - resist decolourisation by acids and alcohols etc.

I have used for this purpose sputum containing numerous tubercle bacilli, a pure culture of human tubercle bacilli on potato (about 9 months old) and pure cultures on agar of the following organisms, obtained from Králo laboratory viz Smegma B. Timothy B. Grass II B. and mist or Dung Bacillus.

Thanks to the kindness of Dr. Leishman, I have been able to examine a pure culture of his acid fast streptothrix.

I have also experimented on impure growths of these organisms which I have obtained viz Smegma B. from prepucial smegma - Timothy grass B from an infusion of phleum pratense - Grass II. B from infusion of hay, potato etc and the Mist-Bacillus from cowdung.

The following is the method I have adopted:-

Films of sputum and of the various cultures were made on slides, not cover glasses. These were allowed to dry in the air, and then fixed by passing through the flame of a spirit lamp in the usual way.

Such films were then heated, and filtered Ziehl-Neelsen's solution was poured on, and allowed to run over the whole slide. The slide was again heated till steam appeared (actual boiling or drying of the solution was always avoided) for a few seconds, and then allowed to stain. The whole staining process lasted 7 minutes.

At the end of this time the film containing the fuchsin was well washed in tap water, and again allowed to dry in the air. It was then subjected to the decolourising reagent.

The Ziehl-Neelsen solution was that ordinarily used having the formula-

Basic Fuchsin 1:
Absolute Alcohol 10:
Sol^{n} of Carbolic Acid 1 in 20 - 100 parts.

In order that the film should be

exposed to the action of plenty of the acid I adopted the following procedure - The decolourising agent was first poured over the whole film, and allowed to rest there a minute or so, in order to remove the greater mass of the stain. At the end of that time, the reagent - acid or alcohol - was poured off, and the slide placed in a wide mouthed bottle containing fresh decolourising solution - The bottle method for decolourising is exactly the same as that which I have adopted for many years for staining films generally, and of blood in particular, and which I have described elsewhere.

In order that each film should be submitted to exactly the same time in fixing, staining and decolourising, in addition to the ordinary films, I made eight such films on the same slide viz - Sputum, culture of human tubercle bacillus, smegma preputiale cultures of smegma, Smithy, Issaeff, mist Bacilli and thiothrix of Bütschleistman.

By this means I was assured that that each was exposed to exactly the same conditions in all stages of the Examination.

Resistance to 25% Sulphuric Acid.

It has been very generally understood, and repeatedly stated in the Text Books, that the tubercle bacillus is very easily decolourised by acids, and that in the ordinary method of staining sputum, care should be taken that the preparations are not subjected too long to the action of the acid.

Mace[3] for example states - "When decolourising by acid solutions it is recommended not to allow the preparation to be submitted to the decolourising agent too long: the bacillus of tuberculosis can in fact be decolourised after an action of 10 to 15 minutes duration. The use of organic acids as decolourising agents easily prevents this mistake."

[3] loc cit p 542.

Abbott and Gildersleeve[46] - referring to the "acid fast bacteria state that as regards resistance to acids probably all members of the group can be at once distinguished from the tubercle Bacillus by the lesser degree of resistance. They retain the stain for a varying time when treated with weak agents (eg 3% HCl in alcohol and 5% H_2SO_4

in water) but are almost at once decolorized by 25 - 30% nitric acid in water.

The same observers[47] in the New York Med. Record - May 1902 - state that "they had found practically the whole group of pseudo tubercle bacilli were almost instantly decolorized, by the usual acid mixtures employed for decolorization, in connection with the examination for tubercle bacilli. This was true not only of the old nitric acid decolorizer but when the now popular 5% (sic, probably means 25%) sulphuric acid was employed." Further they say that "they had found it so easy to differentiate these acid resisting organisms by the old and well known methods that they had not extended their observation to other and new preparations"

[47] loc. cit p. 748.

The results of the following experiments show how absolutely erroneous these statements are.

I find that even the heat of the acid resisting pseudo-tubercle bacilli are not totally decolorized after four hours immersion in 25% sulphuric acid - and much the same in other acids.

Sulphuric Acid. 25%.

Films stained with hot Zehl Nelson and then placed in 25% sulphuric acid, washed, and counter stained in weak methylene blue.

1. At the end of 20 minutes. i Tubricle Bacilli in sputum deeply stained red.
   ii. Tubricle Bacilli in cultures deep red.
   iii Smegma Bacilli in cultures deep red
   iv. Timothy Bacilli deep red.
   v. Grass II. Bacilli a few decolourised. The majority a deep red
   vi. Mist Bacilli deep red.
2. At the end of 1 hour.
   All of the pseuds bacilli stained red.
3. At the end of 2½ hours.
   All or practically all stained red.
4. At the end of 4 hours.
   Tubricle Bacilli in sputum & cultures and Most Bacilli quite red.
   Smegma Bacilli nearly all red.
   Timothy B. most of them red.
   Grass II B. decoloured.
5. At the end of 7 hours.
   Tubricle in sputum and cultures distinctly red.
   Smegma B. many still red.

Timothy B. about ½ red, and ½ blue.
Grass II B completely decolourised.
Mist B. mostly blue but lays patches red.

6. At the end of 9 hours.
Tubercle B in sputum & culture - red.
Smegma B. decolourised
Timothy B. many especially the spherical forms red. the others blue
Grass II B. decolourised.
Mist B. exceedingly well stained red.

7. At the end of 16 hours.
Tubercle B in sputum & culture speedily well stained.
Smegma, Timothy, Grass II and Mist B decolourised.

8. At the end of 24 hours
Tubercle B. in sputum & culture red.
All others decolourised.

9. At the end of 41 hours.
Tubercle B. in sputum & culture red.

10. At the end of 48 hours.
Tubercle B, in sputum ~~& culture~~ some are decolourised but the majority are still well stained red. In cultures they are all well stained

11. At the end of 72 hours. The tubercle B in air

cultures, and in sputum, are clearly stained a brilliant red.

Conclusions:-

a) That at the end of 16 hours all the pseudo tubercle bacilli were decolourised

b) That all resisted the action of 25% sulphuric acid for 2½ hours.

c) That the grass B II is decolourised the Earliest

d) That of the pseudo-tubercle bacilli, the Mist and Timothy B resist the longest.

e) That the Tubercle Bacilli in sputum and in cultures are exceedingly well stained after 72 hours.

Nitric Acid 33⅓%.

Films prepared and stained as before.

1. After 5 minutes:

No decolourisation of any

2. After 30 minutes.

Tubercle B in sputum & cultures well stained
Smegma B - partially decolourised and stained blue -
Timothy B. stained distinctly red.
Grass II stained red.
Mist B. stained distinctly red.

3. At the end of 50 minutes.

Tubercle Bacilli in sputum & culture red.
Smegma B. mostly decolourised but
patches quite red.
Timothy B. about ½ red, ½ blue.
Grass II. B. partially stained, some well
some quite blue.
Mist B. very well stained.

4. At the end of 2½ hours.

Tubercle B in sputum a faint red.
Tubercle B in culture well stained
Smegma B. many decolourised but many
red.
Timothy B. mostly decolourised a few red.
Grass II completely decolourised.
Mist B. very distinct bright red.

5. At the end of 5 hours.

Tubercle B in sputum apparently many
decolourised, some faint, others fairly red.
Tubercle B in culture most distinctly red.
Smegma B. many quite red, but majo-
-rity are decolourised and stained blue.
Timothy B mostly blue. a few red.
Grass II B completely decolourised
Mist B are mostly red. a few
decolourised.

6. At the end of 24 hours - The Tubercle B. and all the pseudo-tubercle B. decolourised.

Conclusions:-

That 33⅓% nitric acid is not adapted for the differentiation of the Tubercle from other pseudo-tubercle bacilli as it soon begins to attack the Tubercle B in sputum - The Mist B is the most resistant of the pseudo-tubercle B.

Alcohol.

It is very generally stated that the smegma bacillus is easily distinguished from the tubercle B - by the fact that the former will not resist decolourisation by alcohol, but that the latter will. Even Kruse in Sluggs's Micro-organisms makes this statement.

Nencki and Podszaski (48) even as recently as this year say that "the smegma bacillus although acid proof has not much resistance against alcohol - If preparations after being stained in the usual way with Ziehl-Nielsen's solution and then treated with acid, are subjected to alcohol - the smegma bacillus will lose its red colour

48. loc. cit. p. 90.

and appears blue when the preparation is after stained with methylene blue."

Nikiten[49] states thay:- 49. loc. cit p. 427.

"1. We do not possess any sure method of distinguishing the tubercle bacillus from all other acid and alcohol proof bacilli.

2. That the presence of different fatty substances in the cell body of acid fast bacteria influences their specific behaviour with Ziehl-Nielsen solution.

3. By "defatting" by ether, alcohol, and xylol the acid fast bacteria lose their resistance against acids. That the tubercle bacillus withstands the "defatting" longest"

Alcohol. 90% Pure Methylated.

Films placed after staining in 25% sulphuric acid for 7 minutes, washed, and then placed in 90% alcohol.

1. After 5 hours.

Tubercle B in sputum 1 culture well stained.
Smegma B. most but not all decolourised some distinctly red.
Timothy B. many quite red, many blue
Grass " many especially in the thinner parts decolorised, but many red.

Mist B. not decolourised at all.

2. After 9 hours.

Tubercle B. in sputum & cultures stained red.
Smegma B mostly decolourised but many stained red.
Timothy B. stained red.
Grass II. B. majority decolourised - blue.
Mist B. majority stained deeply red some blue.

3. After 24 hours.

Tubercle B in sputum and cultures still red but they take on the blue tint and appear darker colour.
Smegma B decolourised.
Timothy B reddish blue distinctly a dark red colour.
Grass II almost entirely decolourised and stained blue. a few red bacilli seen.
Mist B. red colour.

4. After 28 hours.

Tubercle B. in sputum & culture red with tinge of blue. clear and distinct.
Smegma - and Grass II completely decolourised
Timothy B. still reddish blue
Mist B mostly red, some blue.

Conclusion: That alcohol will not

distinguish the pseudo-from the real tubercle Bacilli - that smegma bacillus in cultures resists the action of 90% alcohol - after being treated for 7 minutes with 25% H_2SO_4 for a long time - at least nine hours.

Acid-Alcohol.

Honsell's Method.

For the differentiation of smegma from tubercle bacilli - Honsell's method is recommended by most of the text books - (Hewlett, Lehmann & Neumann).

The former[50] describes this method as follows: 50. loc cit p. 248.

"After staining in warm carbol-fuchsin the specimen is washed, dried; it is then immersed in 3% HCl. alcohol for 10 minutes washed in water and counterstained for a few seconds in saturated alcoholic solution of methylene blue - washed, dried and mounted - The Smegma bacillus is decolorized."

Lehmann & Neumann[2] give practically 2. loc cit p 425
the same directions except that for counterstaining they say wash in alcoholic methylene blue diluted one half.

Horsells Method. 3% HCl alcohol.

1. After 10 minutes:-
   Tubercle B in sputum & cultures stained well.
   Smegma B mostly decolourised but not all.
   Timothy B. well stained red.
   Grass " B. partially decolourised, part especially the thicker distinctly red.
   Rat B. decolourised.

2. After 1½ hours.
   Tubercle B in sputum red. fainter.
   Tubercle B in cultures. distinctly red.
   all others decolourised except Timothy B.

3. After 15 hours.
   Tubercle B in sputum red but fainter.
   Tubercle B in culture well stained red.
   All others decolorized.

4. After 17 hours.
   Tubercle B in sputum faint: in cultures stained red.

5. After 24 hours.
   Tubercle bacilli in sputum practically all decolourised.

Acid Alcohol. 3% HCl in 90% alcohol.

In the following I have counter stained with aqueous solution of methylene blue. but otherwise have used Horsells' method.

1. After 1 hour. Tubercle bacilli in sputum and cultures red.
Smegma B. partially decolourised.
Timothy B. faintly stained red.
Grass II stained red.
Mist B stained red.

2. At the end of 3 hours.
Tubercle B in sputum faint red.
Tubercle B in cultures red, some decolourised.
Smegma B. many well stained red.
Timothy B. completely decolourised.
Grass ii B. mostly stained - some blue.
Mist B. stained red.

3. At the end of 17½ hours.
Tubercle B in sputum fainter red.
Tubercle B in culture distinct red.
The others are decolourised.

4. At the end of 24 hours.
Tubercle B in sputum almost all decolourised. a very few red bacilli seen.
Tubercle B in culture in most cases quite decolourised.

Conclusion:- Stonell's method or 3% HCl alcohol is not a reliable process for the distinction of the pseudo. tubercle from the real tubercle bacilli.

Pappenheim and Frankel's Method.

This method is described by Simon in his Clinical Diagnosis as follows.

"After staining in Ziehl-Nielsen solution, immerse for 3 to 5 times in Frankel and Pappenheim solution, care being taken to let the fluid drain off slowly after each immersion.

Pappenheim solution consists of one part rosolic acid or corallin in 100 parts of absolute alcohol to which methylene blue is added to saturation. This mixture is further treated with 20 parts of glycerine."

I have worked with the following solution.

Ac. Rosolic	0.5
Absolute Alcohol.	50.0
Methylene Blue.	3.0
Glycerine	10.0

[Rosolic acid, or rosaurin is formed by the action of nitrous acid on Rosaniline thus

$C_{20}H_{19}N_3 + 3NOOH. = C_{20}H_{16}O_3 + 3H_2O + 3N_2.$

Rosaniline Nitrous acid = Rosolic Ac.

(Chem. Soc. Journ. 35:159. Dale and Schorlemmer.)

Its chief use is as a dye, but it is also used as an indicator for alkalis. It is fairly soluble in alcohol.]

I have tried Pappenheim's method as described, but found that by pouring on and off the reagent many of all forms of pseudo-tubercle bacilli remained stained. I have therefore determined a time limit at which these are decolourised.

The films after being stained with warm Ziehl-Neelsen's solution are placed in the rosolic acid mixture:-

1. At the end of 7½ minutes.
   Tubercle in sputum and cultures well stained
   Smegma B. mostly blue- many red.
   Timothy B. about ½ red, ½ blue
   Grass " all blue.
   Meat B. nearly all stained red.
2. At the end of 30 minutes
   Tubercle B well stained.
   Most of the others decolourised except
   Timothy bacilli.
3. At the end of 1 hour.
   Tubercle bacilli well stained.
   all the pseudo-tubercle B. decolourised
   and stained blue except a few Timothy B.
4. At the end of 1¾ hours. Tubercle B well
   stained all others decolourised.

5. After 3½ hours -

Tubricle B in sputum & culture well stained red - all others decolourised and blue.

6. After 13 hours.

Tubricle B in sputum and culture stained red - all others decolourised.

7. After 17 hours.

Tubricle B in sputum and culture quite distinct a little darker in colour.

8. After 26 hours

Tubricle B in sputum & culture quite distinct red.

9. After 52 hours

Tubricle B in sputum all stained - most of them distinct red some bluish red but all equally distinct.
Tubricle in cultures red or bluish red.

Papp enheim's Solution without Methylene Blue -

acts very much as the original solution
After 1½ - 2 hours all the pseudo-tubricle B are decolourised - After 24 hours tubercle bacilli in sputum and in culture are distinct and easily recognised.

Conclusions :-

The solution of nucleic acid, alcohol

glycerine, with or without the methylene blue, is not reliable as a differential method for the smegma or other pseudo-tubercle bacilli, when used in the original manner.

After 2 to 3 hours - all pseudo-tubercle B are decolorized by it - whilst the genuine tubercle B - withstands it for 24 to 52 hours.

I therefore consider the mixture when used as I have described, very valuable for distinguishing the Koch's bacilli from all these acid fast bacilli.

The behavior of Smegma Bacilli in their natural medium smegma of the prepuce will be described in detail later.

Bunge and Trautenroth's Method.

This method is described in the text books, (Hewlett and Lehmann & Neumann) as a means of distinguishing acid fast smegma bacilli from the tubercle bacillus.

The methods consists of the following:- (50)

(50) loc cit p 255

1. Film preparations are immersed in absolute alcohol for 3 hours

2. Immerse in 5% chromic acid in water for 15 minutes.
3. Stain in warm carbol-fuchsin 2 to 3 min
4. Decolorise in 25% Sulphuric acid for 2-3m.
5. Counter stain in concentrated alcoholic solution of methylene blue, for at least 5 min.

The smegma bacilli are said to be decolourised.

I found this method entirely useless. After proceeding as described and extending the immersion in 25% sulphuric acid to 7 minutes the following were the results:

i Tubercle bacilli stained red.
ii Smegma bacilli all reddish blue.
iii Timothy B. a few quite red.
iv Grass " reddish blue.
v. Mist B. some quite red in colour.

The process is therefore to my mind, absolutely unreliable for the purpose for which it was intended. It has several disadvantages in addition, it is tedious, the chromic acid must be well washed out before staining, and although I have repeatedly tried the method, I have never got any satisfactory results.

Gram's Method.

1. Tubercle B. in cultures. The effect of Gram's stain on films of tubercle bacilli is not at all unlike that of Neisser - which will be described later - Small deeply stained granules or dots are seen at the ends of most of the bacilli. Many of the shorter bacilli only have the terminal dots, but in the longer forms, there is often a central, sometimes two dots, while in the very long variety of the bacillus seen in old cultures, there is quite a number of dots of equal or unequal size. Sometimes, but more rarely, there is only a central spot. The body of the bacillus is very faintly stained by the fuchsin violet.
2. Smegma B. deeply stained - the bacilli appear rather like young diphtheria bacilli having the appearance of a sheath. There are no granules present.
3. The Smithy, Grass II and Dung bacilli are stained by Gram's method.

The shigo thing of Burt and Leishman is also stained by Gram.

As would be expected, the tubercle B. only stains very faintly with Gram's method.

Neisser's Method.

This is a method devised by Neisser to show the granules in the Bacillus of Diphtheria.

I have subjected the tubercle and the pseudo tubercle to the action of Neisser's stain is acid methylene blue alone - and also to a modification of Neisser's process viz treating the preparation with a solution of iodine, potass. iod and water - after staining with the blue. This modification I found very useful in studying the B. diphtheriæ and described it in full in the Brit. Med. Journal of 1899.

The only organism which I found stains by Neisser is Grass II B.

In films made from cultures of this bacillus the majority were seen to contain deeply stained bluish black granules, but the body of the bacillus did not take on the stain of Bismark brown or safranin as might be expected.

The dots when magnified 2250 diameters is with 1/12 oil immersion apochromatic lense and compensating 18 eyepiece are seen to be fairly equal in size and always round.

Usually there is one dark granule at each extremity of the bacillus, but very often there is a third granule in the centre.

As far as I have seen no other pseudo-tubercle bacillus, nor the acid-fast Diphtheroid of Birt and Leishman contains these granules of Neisser.

Aqueous Solution of Methylene Blue.

When films of the pseudo- and real tubercle bacilli were stained with a concentrated aqueous solution of methylene blue (Grubler's purified) for 10 minutes at the temperature of the room, the following results were obtained.

i. Tubercle Bacilli in sputum were not seen at all and have evidently not taken on the stain

ii Tubercle B in culture. - not stained.

iii Smegma B in cultures well stained. and appear very like young diphtheria bacilli

iv. Grass ", Timothy grass and Mist Bacilli were all stained.

This bears out the statement that the Tubercle B. is not easily stained by aqueous solutions of aniline dyes. All the pseudo-Tubercle B. are stained

A. Ziehl-Neelsen's Stain without Heat. 20 min

Films of the pseudo- and genuine tubercle bacilli were subjected to the action of Ziehl-Neelsen carbol-fuchsin for 20 minutes at the temperature of the room without heat. At the end of this time they were subjected to decolourising action of 25% sulphuric acid. The results were as follows.

1. At the end of 2 minutes in 25% H_2SO_4.
   - i The Tubercle B in sputum were found to be few and those stained faint red.
   - ii Tubercle B. in cultures some distinctly stained, others faint.
   - iii The pseudo-tubercle B. viz Smegma, Timothy, Grass II and Mist Bacilli are all distinctly stained.
2. At the end of 10 minutes in 25% H_2SO_4
   - i. Tubercle B in sputum few seen.
   - ii Tubercle B. in cultures - few and faintly stained some however were quite distinct.
   - iii Smegma B - reddish blue.
   - iv. Timothy B many distinctly red.
   - v. Grass II B - mostly blue.
   - vi Mist Bacilli - most of them are stained a faint red colour.

3. At the end of 40 minutes in 25% H_2SO_4.

i. Tubricle B in sputum + ~~cultures~~ - not seen at all.

ii Smegma and Grass II B - completely decolourised

iii Timothy B. most of them faint red - some distinctly blue - a few distinctly red.

iv. Mist B - reddish pink - not distinct.

v. Tubricle B in cultures many quite red and distinct, some very faint. others decolourised.

Evidently from this it would seem, that the tubricle Bacillus is not as easily stained with an anilin dye, and a mordant as the pseudo-tubricle bacilli

B. Lichl-Neelsen's Stain without heat - 1 hour.

Films of the pseudo- and real tubricle Bacilli stained with Lichl-Nielsen's Carbolfuchsin for 1 hour - at the temperature of the room without heat. Decolourised in 25% Sulphuric acid:- counterstained with weak Methylen Blue.

1. After 15 minutes in 25% H_2SO_4.

i. Sputum - very few tubricle bacilli found but these are clear red.

ii Tubricle B in cultures well stained red.

iii Smegma B. some well stained red, most of them are purplish colour.

iv. Timothy B. mostly red, some blue.

v. Grass II. B. nearly all decolourised, a very few red

vi. Mist B. quite red.

2. After 3/4 hour in 25% H_2SO_4.

i. Tubercle B in sputum - very few found.

ii. Tubercle B in cultures most of them well stained.

iii Smegma B. decolourised - but slightly purplish

iv. Timothy many stained red.

v. Grass II completely decolourised.

vi. Mist B. quite red.

3. After 3/2 hours in 25% H_2SO_4.

i. Tubercle B - none found in sputum.

ii Tubercle B in cultures visible - but faint.

iii all the pseudo-tubercle bacilli decolourised.

c. Ziehl Neelsen's stain without heat. 1 hour.

Films stained with Carbol fuchsin for one hour at the temperature of the room, and placed in the Erodic acid mixture (Pappenheims sol^n).

1. Pappenheims Solution with methylene blue - for 1/2 hour.

All the pseudo-tubercle B decolourised - and only a few of the tubercle B in sputum and culture are stained red.

2. Pappenheim's Solution without methylene blue. practically the same results as above.

Films previously treated with Alkalies.

Mace[3] has stated that "the Bacillus of smegma may under certain conditions resist decolourisation by nitric acid. This is particularly the case when it is impregnated with grease. By treating the preparation for 10 minutes with "une lessive de soude additionée de 5/100 d'alcool" one removes the grease and at the same time the power of resisting decolourisation. Under the same conditions the B of tuberculosis retains its stain after the action of the acid."

3. loc. cit. page 508.

In order to ascertain whether this statement was of any practical value I made the two following series of Experiments:-

I. Films previously treated with Liq. Potassae B.P. for 10 minutes.

Films of the pseudo and real tubercle bacilli were after being fixed in the usual way placed in liq potassae for 10 minutes; washed, dried and then placed in ether for 5 minutes. They were then stained with hot carbol-fuchsin in the usual way.

I found after this treatment, that some of the acid resisting power was diminished.

II. I further subjected films dried in the air, and fixed by heat, to the action of the following solution

Aqueous Sol^{n} of Caustic Soda 1 in 12 – 40 parts

Alcohol – 90% 10 parts.

for ½ hour. Then washed, dried and stained. Them in the usual way with warm Loehl-Neelsen's carbol-fuchsin, (4 minutes) and after again washing and drying – placed them in 25% sulphuric acid. The following were the results:-

1. At the end of ½ hour.

i Tubercle Bacilli in sputum & culture well stained red.

ii Smegma (Cultures) B. nearly all red

iii Timothy B some still red.

iv. Grass & Meat B. well stained.

v. Smegma (preparation) Bacilli well stained

2. At the end of 1½ hours –

i. Tubercle B in sputum and culture stained red.

ii Smegma B in cultures many stained red.

iii Timothy B. many smaller forms still red

iv. Grass & and Meat B a few cocci forms red.

v. Smegma preparation, many of the bacilli stained distinctly red. I shall refer to the staining reaction of smegma in detail later.

3. At the end of 12 hours.

i Tubercle B in sputum and culture well stained red.

ij Smegma B in culture practically all decolourised except a very few cocci forms.

iij Timothy B - a few distinctly red.

iv. Grass II. B. almost entirely decolourised a small patch of red bacilli seen.

v. Meat B. a very few quite red, most blue.

vj Smegma pulpatiab - much of the background remains red, but no very evident bacilli stained red seen.

Conclusions:

From these experiments, it is very evident that previous treatment of the films with strong alkalies, have very little effect on the subsequent resistance to acids of any of the pseudo-tubercle bacilli.

That the smegma bacilli in cultures, and in smegma itself after such treatment resist the action of 25% sulphuric acid for at least 2½ hours - and at the end of 12 hours are not thoroughly decolourised.

That previous treatment of a film with strong alkalies tends to clear off the film, especially of sputum - and in the case of milk or urine, would be therefore impracticable

The Smegma Bacillus as seen in Smegma.

Having described the characters and power of resisting acids, alcohol &c which the Bacillus Smegma in cultures possesses, I have made some further inquiry into the behaviour of the bacilli in their natural medium i.e. smegma.

For this purpose I have used entirely human preputial smegma, and in my experiments found that the three following conditions are met with:-

1. That the number of bacilli smegma in preputial smegma varies very greatly. In some specimens they are present in enormous masses, whilst in others they are very scanty.

I found that in the smegma of adults the bacilli were, at least in some cases very few in number, whilst in that of a young girl aged about 7 years they were exceedingly numerous.

2. That the Bacillus smegma as met with in smegma behaves very differently with regard to its power of resisting acids, alcohol &c.

In some smegmas, especially that obtained, without the use of antiseptics, from cases

of circumcision, and from adult males, the bacilli were almost immediately decolourised by 25% sulphuric acid - in fact I found it was impossible in these particular cases to obtain good specimens - After the action of 25% sulphuric acid for a few seconds, ~~or less~~, very few bacilli stained red were seen. This might of course have been because they were not present in the film, but on preparing another film and using 12½% sulphuric acid for a very short time - a few seconds - I found them very numerous. By examining such a preparation mounted in water, and having 'logged' the position of a group of bacilli by means of the graduated mechanical stage - I removed the coverglass and tried the effect of simply pouring 25% sulphuric acid on and off once. On again examining the same patch I found that very many of the bacilli were ~~were~~ previously red were completely decolourised by this treatment. This feeble resistance to acids may be due to the presence of much fatty matter in the film preventing the action of the Ziehl-Neelsen's fuchsin, and therefore the organisms being faintly stained are easily decolourised,

or it may be that the smegma bacilli may under different conditions - which are at present unknown, behave very differently in their resistance against decolourising agents.

The possible occurrence then of feeble acid resisting smegma bacilli, may explain the statement previously expressed by many writers, of the ease with which the Smegma bacillus is decolourised.

3. That in the preputial smegma taken from a young girl I found the smegma bacilli very much more resistant to acids, alcohol etc than in the above mentioned cases.

These resisted decolourisation by acids and alcohol as long as any those in cultures, and nearly as long as any of the other pseudo-tubercle bacilli with which I am familiar

The Differential Diagnosis of Smegma from true tubercle Bacilli is of the greatest importance. This is especially so of the genito-urinary tract, and acid fast bacilli found in this region can not be declared true bacilli of tuberculosis.

Moeller[16] in doubtful cases differentiate

[16] loc. cit. p. 46

these pseudo-tubercle bacilli by a simple method, depending on the fact of the slow growth of the tubercle bacillus, and the higher temperature required for its growth.

He mixes the secretion to be examined with nutrient bouillon, and keeps it in the incubator at a temperature of 28° to 30°C. If in the course of a few days there is a visible increase in the bacteria resistant to acids, one can assume with certainty that the case is not one of true tubercle but pseudo-tubercle.

The true tubercle bacillus requires a temperature of 37° for its growth, and if mixed with other bacteria would be overgrown by them, before any increase could have taken place - owing to its slow growth.

"Sometimes (35) when the sputum is mixed with certain nutrition media, the tubercle bacillus grows at incubation temperature. This proliferation due in all probability to the importation of globulin-like substances from the body, is however exceedingly small, and ceases altogether after, at the latest 48 hours, whilst in the pseudo-tubercle bacilli a persistent further proliferation takes place at 30°C."

35 loc. cit p. 491

The following are the results of my experiments on Smegma Bacilli in Smegma.

Sulphuric Acid - 25%.

Films of preputial smegma, fixed by heat and stained by Ziehl-Nielsen's solution exactly in the manner described previously, were immersed in 25% sulphuric acid, well washed and counter stained with weak watery solution of methylene blue:-

i. At the end of 15 minutes - bacilli well stained
ii " 30 " " " "
iii " 70 " " " "
iv. " 1½ hours - most of the bacilli stained
v. After 2½ hours - many of the bacilli decolourised but some are still distinctly stained.
vi. After 4 hours - a few of the bacilli are still stained clearly red although most are decolourised.
vii. At the end of 8 hours - Still a few bacilli especially in the thicker part of the films red.
viii. At the end of 9½ hours - very few bacilli red chiefly the spherical forms.
ix. At the end of 13 hours - all decolourised -

It is interesting to notice that the form of the smegma bacillus which resists

the action of the acid for the longest period is that of the spherical - thick short shaped organism - and this seems to be the case in cultures of this and the other pseudo-tubercle bacilli.

Conclusions: That the smegma bacillus in smegma resists decolourisation with 25% sulphuric acid for a considerable time - at least ½ an hour, and in this way agrees entirely with the results I have obtained with these bacilli in pure cultures.

Alcohol 90%.

Films stained with carbol. fuchsin in the usual way, then placed in 25% sulphuric acid for 15 minutes, washed - dried and placed in 90% alcohol.

i. At the end of 30 minutes - smegma bacilli in smegma not decolourised

ii. At the end of 1 hour - many decolourised.

iii. At the end of 2 hours - all smegma bacilli decolourised.

Rosolic Acid Mixture - Pappenheim Solution -

Films of smegma - stained with carbol fuchsin in the usual way - were placed in rosolic acid mixture - and were found to be decolourised at a period differing from

1/2 to 2/2 hours - The spherical forms resist the longest.

Rosolic acid mixture without the methylene blue, seems to act somewhat quicker and better than that with methylene blue.

Acid Alcohol - 3% HCl in 90% Alcohol.

Films of smegma - stained with Licht Nielsen's solution in the usual way. The bacilli are completely decolourised at the end of 30 minutes.

Nitric Acid 33/3%.

Films stained with Licht. Nielsen as before. After 40 minutes nearly all decolourised - but the back ground still red, and a few red bacilli seen.

After 60 minutes practically all the smegma bacilli are decolourised.

Previous treatment with Alkalies.

Films of putrefied smegma dried in the air, and fixed by slight heat - were placed for 15 minutes in

Aqueous sol^{n} of Caustic Soda. 1-12. 40 parts.
Alcohol. 90%. 10 parts.

i. At the end of 4 hours. many of the smegma bacilli exceedingly well stained.

ii At the end of 8 hours. a few bacilli stained.

especially the spherical cocci forms.

iij At the end of 13 hours all decolourised.

These results are practically the same as I found with Smegma bacilli in pure cultures (see page. 108).

Comparing the results obtained when films are fixed by heat, with those when previously treated with soda in alcohol - I should say that the latter treatment does not render the smegma bacillus easily decolourisable - and it is not a method for distinguishing the smegma from the genuine tubercle bacilli as Macé would have us believe.

Resistance of the Acid-fast Streptothrix of Birt and Leishman.

I will very briefly state the results which I have noted as regards the resistance to alcohol and acids which this streptothrix possesses.

Birt and Leishman (42) state that "the acid-fast nature of these two forms - (the threads and arthrospores) varies with the age of the cultures - Klein and Moeller have noted similar features in old cultures of Tubercle and Grass Bacillus ||

42 loc cit. p 126.

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 decolourised. On the other hand the arthro-spores remain acid fast for as long as we have had them under observation".

My experiments bear out this statement. The threads are easily decolourised the spores not nearly so readily give up their stain.

Sulphuric Acid 25%.

In films made from a 3 days old bouillon and agar culture - some of the threads lose their red stain after the acid has been poured on and off - and take on the counter stain - In a film prepared as sputum would be all the spores - cocci - bacilli - are stained red clearly - and some of the filaments, but some of the latter are coloured blue by the after-staining with methylene blue.

In an agar culture one month old there are fewer threads - and more cocci - bacilli or oval and rod shaped bodies present - and here the threads are seen less resistant -

i. At the end of ½ hour. The rods, and cocci forms are not decolourised.
ii. At the end of 1½ hours. still red.
iii. At the end of 2½ hours. still red.
iv. At the end of 6½ hours. all the cocci forms are red.
v. At the end of 12 hours all decolourised.

Rosolic Acid Mixture: Pappenheim's solution.

i At the end of 30 min the cocci forms are not decolourised.
ii At the end of 1½ hours. nearly all the cocci forms are decolourised + blue.
iii At the end of 3 hours all completely decolourised.

Acid Alcohol. 3% HCl. in 90% Alcohol.

At the end of ½ hour all forms are completely decolourised.

Rosolic Acid Mixture without Methylene blue decolourises all forms of the acid-fast Streptothrix in ½ to 1 hour. and seems to act better than with methylene blue.

Conclusions: The threads of the Streptothrix are only moderately acid-staining. The so-called spores are nearly as acid resisting as the other pseudo-tubercle B.

General Conclusions.

1. That the tubricle bacillus is not the only acid and alcohol-resisting bacillus.

In addition to the bacillus of leprosy several other acid and alcohol fast bacteria are known. Amongst these are the Smegma bacillus, Timothy grass, Grass II, meist, butter bacillus and one or two species of the streptothrix.

These acid-fast organisms form a distinct group, some of which appear to be saprophytic, eg Möller bacilli, some eg tubricle bacillus are facultative parasite, whilst the bacillus of leprosy is an obligate parasite.

2. That the acid resisting property depends upon the presence of a waxy substance in the body of the bacillus.

This is found in the pseudo- as well as in the bacillus of tuberculosis.

3. That the tubricle bacillus can withstand the decolourising action of acids and alcohols for a very long time, very much longer than is generally stated.

4. That the acid-fast pseudo-tubricle bacilli

resemble the genuine tubercle bacillus not only in their acid-fast character, but also in their size and shape and in the fact that they tend, at least in many cases, to produce in animals nodular diseases, in which giant cells etc are found.

It is probable that the pseudo- and genuine-tubercle bacilli are really members of the achromomycotic group of fungi.

5. That although the pseudo- and genuine tubercle bacilli are all acid-fast - yet they are not equally resistant. The tubercle bacillus withstands decolourisation by acids and alcohol, considerably longer than ~~of~~ the other acid-fast organisms with which I have experimented.

6. That the pseudo- and genuine tubercle bacilli cannot safely for diagnostic purposes be differentiated by their morphological characters. The microscopical distinction by the hitherto described methods, is in all cases difficult, and in many impossible.

7. That the various differential methods of staining these organisms described in

textbooks are according to my experiments absolutely erroneous.

8. That any staining method for differential purposes must depend on the fact, that the tubercle bacillus is much more acid resisting than any of the pseudo-tubercle bacilli. It is therefore necessary to determine the earliest time at which all the pseudo-tubercle bacilli are decolourised, and how long on the other hand the bacillus of tubercle can safely resist the action of the decolourizer; and then to ascertain whether the results so obtained are constant.

9. That amongst other results the experiments which I have made show that the genuine tubercle bacillus can resist the action of 25% sulphuric acid for at least 72 hours, whilst all the pseudo tubercle bacilli are completely ~~destroyed~~ decolourised at the end of 16 hours, many before that time.

That the tubercle bacillus in sputum and in cultures resists the decolourising action of the Rosolic acid mixture well till at least 52 hours, whilst all the pseudo-tubercle bacilli are decolourised

at the end of 3 hours - and most of them considerably before that time.

From these results I would formulate the following method for distinguishing the pseudo- from real tubercle bacilli.

Differential Method - for differentiating all acid-resisting bacteria from Tubercle Bacilli.

i. Spread films of sputum, milk, urinary sediment, or the fluid thought to contain tubercle bacilli, on slides, taking care to make the preparation as thin and uniform as possible.

Dry in the air.

ii. Fix by passing through the flame of a spirit lamp in the usual way.

iii. Whilst still ~~hot~~ warm pour on filtered Ziehl-Nielsen's carbol-fuchsin, allowing it to spread over the slide. Let this stand for 1 minutes. Again warm the slide over the spirit lamp for a few seconds - taking care to prevent actual boiling of the stain.

Then allow it to stand and stain for about 7 minutes.

iv. At the end of this time thoroughly wash

and decolourise in either of the following ways.

(a) In Rosolic Acid Mixture :-

Ac. Rosolic. 0.5 parts.
Absolute Alcohol 50.0
Methylene Blue. 3.0
Glycerine 10.0

On the film stained as above pour a few drops of this solution and then let it drain off. and place the film then in a wide mouthed bottle, containing this solution for at least 4 hours. and not longer than 24 hours.

After an immersion of 4 hours, all the pseudo-tubercle B are obtained blue. the bacillus of tuberculosis remains red.

I found it advantageous to omit the methylene blue in the above mixture, and prefer to counterstain the film for a minute or so in any watery solution of methylene blue. By this means the tubercle B remain a very brilliant red colour.

β. In Sulphuric Acid 25%.

Pour on a few drops of the acid on the stained film, and after letting it act for a minute, pour off. and then place the slide

in a wide mouthed bottle containing 25% H_2SO_4 for at least 16 hours, but not longer than 24 hours. Wash thoroughly, and counter stain with a weak watery solution of methylene blue.

Any organism which remains stained red after treatment by either of these acids for the time stated, is in my opinion the true bacillus of tuberculosis.

10. In cases in which, after using the differential staining method just described, doubt exists as to the organisms under consideration being the genuine tubercle or pseudo-tubercle bacilli - two other methods should be resorted to - 1st the cultivation and 2nd the inoculation.

1st Cultivation - The excretion to be examined is mixed with nutritive bouillon and incubated at 30° C. If within two or three days there is a visible increase in the bacteria resistant to acids, it is certain that they are not the genuine tubercle bacilli. The bacillus of Koch requires a higher temperature and a longer time for its growth. This method has however many practical difficulties.

2nd Inoculation. An animal known to be very susceptible to tuberculosis eg the Guinea pig - is injected intra peritoneally with the secretion to be examined.

After a month to 6 weeks if the animal lives as long it is killed and examined.

This method is conclusive, but it has the disadvantages, that the time required before a result can be obtained is long, and that a special license is required.

The former is a serious objection to the Medical Officer of Health - as the Food and Drugs act requires that proceedings shall be taken within one month.

As one speaker at the British Congress of Tuberculosis held in London in July 1901. says - "What the bacteriologists are sighing for is some method which will enable us to decide in three weeks, whether the particular organisms found (in milk for example) are the Tubercle bacilli or not. Nothing less will satisfy us."

I think that the differential staining method which I have described will meet that want, but only time and further confirmation will

prove whether it is constant and reliable.

My experimental work has proved conclusively that the usually described methods for the differentiation of the pseudo and genuine tubercle bacilli are absolutely fallacious, and if as I think the method which I have described proves as reliable and constant in other hands as it has in mine - we are certainly in a much better position to distinguish within a short time these harmless groups of organisms and the cause of such havoc to the human race - the bacillus of tuberculosis.

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