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CLINICAL

DIAGNOSTIC BACTERIOLOGY.

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DIAGNOSTIC BACTERIOLOGY

INCLUDING

SERUM DIAGNOSIS & CYTODIAGNOSIS

BY

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F.R.S. Edin.

WITH COLOURED PLATES

PREFACE.

WITH so many excellent works on Bacteriology now obtainable, it may seem necessary to give some reason for the production of a small book with such an ambitious title.
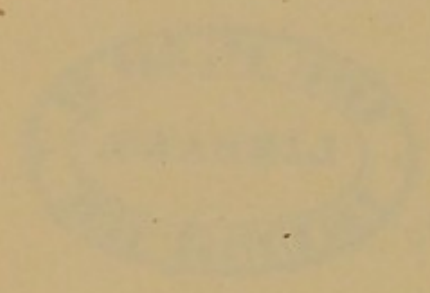
Many practitioners who have not had an opportunity of studying in a laboratory, or who may not have the necessary equipment for such work, are still anxious to avail themselves of some of the advantages to be derived from a bacteriological examination.

The questions which occur to them are: How shall we obtain the material; how shall that be prepared for microscopic examination; what are the chief characteristics of the organism in question; with what may it most easily be confused; how can it be distinguished; and, lastly, what inferences can be drawn from such an examination which will help us in making a diagnosis, prognosis, or in carrying out treatment?

Most of the existing works on bacteriology treat the subject from a laboratory point of view, and although many helpful suggestions are given as to the clinical diagnosis, yet the subject is not especially written for this end.

In the following pages I have endeavoured to answer these questions, and to place before the reader the methods of examination best adapted for practical diagnostic purposes.

I have included an account of some research work which I made on the "Acid-fast Bacteria: their

resemblance to, and differentiation from, the Tubercle Bacillus," which appeared in several numbers of the *Journal of State Medicine*. This is a subject which is attracting considerable attention, and is, up to the present, but inadequately dealt with in the textbooks.

I have also given a short account of Serum Diagnosis and Cytodiagnosis, in so far as they are within the reach of the general practitioner.

Such bacteriological examinations as require very elaborate investigations, *e.g.*, cholera, glanders, etc., I have entirely omitted.

I have already, in "The Blood : How to Examine and Diagnose its Diseases," considered the subject of malaria, and in the third edition hope to include there an account of the trypanosoma, as both of these subjects seem closely associated with the study of the blood.

I have made free use of most of the excellent textbooks written in English, and am particularly indebted to that monumental work, which has already run into three volumes, the " Handbuch der pathogenen Mikroorganismen," edited by Kolle and Wassermann, as well as to " Die Mikroorganismen " by Flügge.

I have also extracted largely from the works in French of Macé, and also Wurtz.

I have endeavoured, in all cases which were open to differences of opinion, to give the name of an authority.

YORK HOUSE,
    BOURNEMOUTH.

CONTENTS.

COLOURED PLATES.

CLINICAL
DIAGNOSTIC BACTERIOLOGY.

Methods of Spreading, Fixing and Staining Films.

THE method of preparing and staining films for microscopic examination will be described under the various organisms which are to be examined, but there are certain details, on which largely depend the success of such an examination, which may be briefly considered here.

Coverglasses.—These should be the thinnest, generally known as No. 1, and either round or square, about $^3/_4$ to $^7/_8$ inch in diameter. When films are made on the coverglasses it is more convenient to use square ones, they are also slightly cheaper, and I think somewhat less likely to break.

There are various methods of cleaning new coverglasses. The plan I use is to drop them one by one into a wide-mouthed bottle containing Van Ermengen's solution, which consists of 6 parts of strong sulphuric acid, 6 parts potassium bichromate, and 100 parts of water. Ordinary battery solution does very well. In this they are allowed to remain some time. I generally leave them for 2 or 3 days, although it is said they become brittle after a time. They are then thoroughly washed in running water, and are kept in little wide-mouthed glass jars or bottles in alcohol.

Instead of the above solution, they may be placed singly in strong sulphuric or acetic acid for 1 hour, and then well washed.

Slides, if new, may be easily cleaned with soap and water, thoroughly washed, and dried with a clean linen handkerchief.

Old slides and coverglasses, if mounted in balsam, should first be heated and the coverglass removed, and both of them dropped into the bichromate mixture and treated as before. If there is much balsam on the slide this may be readily removed by means of a little xylol.

If slides or coverglasses are left for a long time in a strong alkaline solution, they become misty. Wright* says that for blood films the surface of the glass should be rough, that the polishing of the slides is responsible for the patchiness of the blood film, and he advises that the slides should be boiled in strong caustic potash till the surfaces become definitely hazy, which will disappear when oil or balsam is placed on the film.

As most of the films are merely examined in oil without a coverglass, I remove this after the examination is completed with a clean rag, and drop the slide into a stoppered glass jar containing lysol, soap and water, and let them remain there till they are cleaned, either as above or directly with soap and water, or by boiling them with soap and a little soda, and after rinsing well in running water, dry them.

Spreading Films.—The material most frequently examined will be sputum, pus, blood, mucus, the sediment from various excretions and exudations, and the method of spreading films from these will be described later. The usual fault is that the films, which are really

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* Wright: *Lancet*, Jan. 23, 1904.

smears, are too thick. One should try and obtain a uniform and thin film.

I have, throughout, spoken of films made on slides, as never, by any chance, do I use coverglass preparations. Coverglass films of sputum, pus, blood, etc., are made by placing a drop of the material on one coverglass, covering this with another and gently sliding them apart.

Films should always be allowed to dry in the air before being fixed; this may be hastened in most cases by slightly warming the slide or coverglass over a flame, or in the case of blood films, by waving them in the air.

They may be *fixed* by holding them over a flame from a spirit lamp, or in coverglass preparations by passing them through the flame three times. Heat answers very well for most preparations, but if it is desired to fix blood films, or if the film is made from a sediment—such as urine—which is readily washed off, or if one wishes to study the structure of the organism, *e.g.*, the polar staining of the plague bacilli, they should be fixed by immersion in absolute alcohol, or in a mixture of equal parts of alcohol and ether, for a few minutes (any time from 1 to 30). In many cases, for practical purposes, it will be sufficient to allow the film to remain for a few seconds, especially if the alcohol is allowed to evaporate and is not washed off.

Staining. Bacteria are stained by the basic aniline dyes. In order to increase the staining power mordants are often added to the solution of the dye, *e.g.*, carbolic acid in Ziehl-Neelsen's solution, and aniline water in aniline water gentian violet.

The formula of many of the special stains will be

found in the text. I will only here mention those of almost universal use.

LÖFFLER'S METHYLENE-BLUE.

Saturated Solution of methylene-blue in alcohol   30 cc.
Solution of potassium hydrate in distilled water
(1 in 10,000) .   .   .   .   .   .   100 cc.

"This dilute solution may be conveniently made by adding 1 cc. of a 1% solution to 99 cc. of water."— (Muir & Ritchie.) Stain film preparations for 2 to 5 minutes, or less if heat is used. It does not readily overstain.

KUHNE'S METHYLENE-BLUE.

Methylene-blue   .   .   .   .   .   1·5 grams.
Absolute Alcohol   .   .   .   .   .   10 cc.
Carbolic Acid Solution (1 in 20) .   .   100 cc.

Stain in the same way as with Löffler's solution.

Ziehl Neelsen's Carbol-fuchsin is used for staining the tubercle and other acid-fast bacilli. When diluted, 1 in 10 with water, it may be used as a general bacterial stain. The formula will be given later.

CARBOL-THIONIN-BLUE.

Dissolve 1 gram of thionin in 100 cc. of 1 in 40 carbolic acid solution. For use dilute 1 volume with 3 of water. It is more permanent than methylene-blue. Stain for 3 to 5 minutes, or longer; overstaining does not easily occur. Sections should be well washed with water after staining.

The formula for Nicolle's carbol-thionin is given somewhat differently in various text books.

Concentrated alcoholic solution of Thionin in
50 % alcohol .   .   .   .   .   .   10 cc.
Carbolic Acid solution, 1%   .   .   .   100 cc.

is the formula given by Friedberger.

I have always used the simple 1% carbolic solution.

I usually keep the solutions of stains in three different ways. 1st.—Those that readily form a sediment and which require to be always filtered before use— *e g.*, carbol-fuchsin, in bottles fitted with a small funnel, in which is placed a piece of filter paper. 2nd.—Those which do not readily form a sediment and which are clean stains, and this includes almost all those in common use, I keep in wide-mouthed bottles fitted with ground-glass stoppers (Fig. 1). (They were made for me by the York Glass Company). These may also conveniently contain the fixing solution, *e.g.*, alcohol and ether, or the decolourising fluids, *e.g.*, 25% sulphuric acid. Sometimes a scum forms on the surface of the stain and this may stick to the glass slides, but can be obviated, as Daniels has suggested, by thoroughly shaking up the bottle containing the stain before the films are placed in it. These wide-mouthed bottles hold at least two slides back to back, or even a pair of such. The decolourising solution, such as 25% sulphuric acid, will be removed fairly frequently, but

FIG. 1.

most of the stains and fixing reagents will retain their action for a very long time, especially if the stopper is always replaced at once. It may be necessary to filter the solutions, and add some fresh stain occasionally.

Fixing, staining, and decolourising by the "bottle method" is economical, cleanly, and entirely satisfactory. 3rd.—A few solutions which are only used in small quantities, and which deteriorate if allowed to evaporate, particularly Jenner's and Leishman's methylic
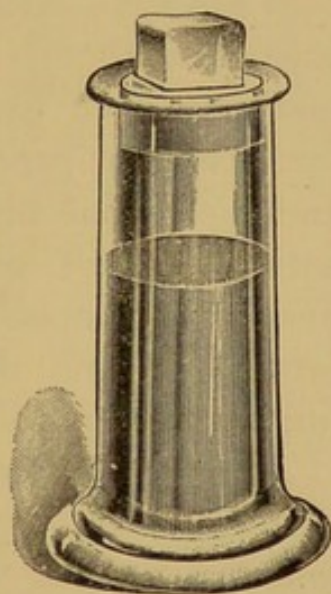
alcoholic solutions of stains, may, with advantage, be kept in a Chalk's bottle, or in a bottle fitted with a pipette stopper and rubber teat.

All stains should be made with distilled and not tap water, and should preferably be made with Grubler's aniline dyes.

Burroughs, Wellcome & Co, have placed on the market 'soloid' forms of the aniline dyes for microscopic purposes. Of these I have had no experience except with Leishman's stain, which I found very satisfactory.

Washing.—After a film has been stained it may be washed with tap water, except when delicate staining methods have been used. Distilled water is essential for washing preparations doubly stained with eosine and methylene-blue, with Jenner's and Leishman's, and with Neisser's original method. It was only after very many months indifferent results with eosine and methylene-blue double staining, that I found out the importance of this fact.

Drying.—After the films have been stained and washed, they may be dried by (i.) waving them to and fro in the air, (ii.) by gently warming over a flame, or (iii.) as is so generally recommended, by blotting them with a piece of filter paper. The first method is satisfactory; the second will do generally, but it usually causes some fading of the methylene-blue, especially when double-staining has been used, and is absolutely to be avoided when films have been stained with Romanowsky's method or Leishman's modification of it. The third procedure I have never used, probably merely from prejudice, but I fancy that it may, especially in blood films, rub or displace the elements, or may leave fluffs, whilst in a film which

is not too firmly fixed to the slide, *e.g.*, a urinary sediment, I am afraid it may detach part of it.   I am convinced that a film, at least one delicately double-stained, should not be allowed to dry slowly in the air because the nuclear staining tends to become fainter by the slow evaporation of the water, and it is essential to hasten drying by some means.   I find the best plan of all is to shake off the water on the surface of the slide, and blow on the film with a Politzer's bag, and dry the under surface of the slide with a linen handkerchief.

Mounting.—When the stained film is quite dry it may be mounted in Canada balsam in xylol, and a permanent preparation made.   It is advisable to use Grubler's neutral balsam in xylol as all aniline stained preparations, especially those stained by Jenner's and Leishman's stain, retain their colour longer.

It is usually said to be advisable to clear the preparation with xylol before mounting in balsam, but although this may improve the clearness of the film I am not satisfied that I can detect much difference. It certainly allows the balsam to spread out more readily, but it then takes a little longer for the cover-glass to set.

Generally speaking, for diagnostic purposes one can dispense with a coverglass and balsam, and merely put on a drop of immersion or thickened cedar oil, and examine directly in this medium.   When the preparation has been examined, the oil should be removed and the slide set aside for future cleaning.

If it is desired to keep the preparation the oil may be removed by a few drops of xylol, and then mounted in balsam with a coverglass.

Gram's Method and its Modifications.

This is one of the most important staining reactions for bacteria, which are distinguished as those which stain by Gram, *i.e.*, retain the colour whilst the tissues lose it, and those which do not stain by Gram but give up their colour when subsequently treated with a solution of iodine and alcohol.

In the former case the bacteria are stained with gentian violet, whilst the tissues are stained with the after stain; in the latter, the organisms as well as the tissues are stained with the second stain.

The ordinary method of staining with Gram is as follows :

1.   The film is stained with aniline water gentian violet for about 5 minutes, and then washed in water.

2.   The preparation is then treated with Lugol's solution of iodine, *i.e.*, iodine 1 part, potassium iodide 2 parts, distilled water 300 parts, till it becomes a purplish black colour, generally in about 1 to 2 minutes.

3.   Decolourise in absolute or methylated alcohol until the colour has almost gone, or the preparation is of a faintly lilac tint—generally in about 10 seconds.

4.   Wash in water and counterstain with a saturated solution of Bismark brown in water, or with carbol-fuchsin diluted 1 in 10, or with a watery solution of eosine or safranine.

5.   Wash, dry and mount in oil or balsam.

The aniline water gentian violet solution is made from two solutions.   First—aniline water is made by shaking up about 5cc. of aniline oil with 100 cc. of distilled water.   This is filtered and kept from the light in a covered bottle.   Second—a saturated solution

of gentian violet in alcohol is made. For use, 10 parts of the aniline water are added to 1 part of the alcoholic gentian violet solution, and the mixture filtered. This preparation has the disadvantage that it must be freshly made, as it will not keep. Carbol gentian violet, consisting of 1 part of a saturated alcoholic solution of gentian violet, and 10 parts of 1 in 20 carbolic acid solution made with distilled water, acts just as well and will keep. *Carbol Thionin*, made of 10 cc. of a saturated solution of thionin in 90% alcohol, added to 100 cc. of a 1% solution of carbolic acid, may, according to Hewlett,* be used for Gram's method. Preparations are stained in this for 2 to 3 minutes, and then treated with an iodine solution somewhat stronger than Gram's, *viz.*, iodine 1, potassium iodide 2, water 200 parts. After remaining in this solution for 2 to 3 minutes, they are decolourised in alcohol containing 1% of acetone—methylated spirit does very well, and subsequently treated as in Gram's method.

CLAUDIUS' MODIFICATION OF GRAM'S METHOD.

1. Film preparations are stained in a 1% watery solution of methyl violet for one minute.

2. Washed in water, and dried with filter or blotting paper.

3. Place in a half-saturated aqueous solution of picric acid for one minute. Wash in water and dry with filter paper.

4. Decolourise film preparations in chloroform, sections in clove oil.

5. Dry and mount in oil or balsam. Films may be counterstained with lithium carbonate or Bismark brown.

* Hewlett: *Manual of Bacteriology*, 1902. 2nd Edition, p. 92.

Cladius' method gives the same result as that of Gram, with the exception that the bacillus of malignant œdema and symptomatic anthrax, are both stained by the former and not by the latter method.

WEIGERT'S MODIFICATION OF GRAM'S METHOD.

Film preparations are treated in exactly the same way as in Gram's method, except that after the action of iodine, instead of being decolourised in alcohol, they are washed in xylol, then dried and after-stained with dilute carbol-fuchsin (1 in 10). They are again washed, dried, and mounted in oil or balsam.

THE FOLLOWING ORGANISMS ARE STAINED BY GRAM'S METHOD.

The Bacillus of anthrax, tubercle, leprosy, tetanus, diphtheria, strepto- and staphylo-cocci pyogenes, micro-coccus tetragenus, diplococcus pneumoniæ (Fränkel), the bacillus of rhinoscleroma, mouse septicæmia, and the mycelium of actinomycosis.

THE FOLLOWING ORGANISMS ARE NOT STAINED BY GRAM'S METHOD.

Bacillus pneumoniæ (Friedländer), influenza, plague, gonococcus, meningococcus, spirillum of relapsing fever, cholera and fowl cholera, bacillus of typhoid, coli communis, glanders, Malta fever, and the bacillus of soft sore. The clubs in human actinomycosis are not stained ; those in animals are.

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It is hardly necessary to add that a $\frac{1}{12}$ inch oil immersion is essential for bacteriological work. This need not be an apochromatic, as a good achromatic lens will answer all purposes, and I can most strongly recommend Leitz' $\frac{1}{12}$ inch objective.

A point not sufficiently realised is the need of a suitable condenser. Mr. E. M. Nelson, than whom there is no greater authority on microscopic lenses, in a private communication, says : " It is essential that the condenser be achromatic, and of a power somewhere near that of the objective in use. It is found that a condenser about $\frac{1}{6}$ inch in power is best for an oil immersion of $\frac{1}{10}$ or $\frac{1}{12}$ inch lens. Zeiss provides nothing but a very low power achromatic condenser. As to the aperture of the condenser, a dry condenser of N.A. 1.0. is sufficient for a Leitz $\frac{1}{12}$ inch objective of N.A. 1.3. A condenser suitable for this lens is Watson's dry parachromatic N.A. 1.0."

At my request, Messrs. Krohne & Sesemann have made a small metal box for carrying microscopic slides in as small a space as possible, without permitting adjacent slides to touch (Fig. 2.) It measures just over 3 inches in length, 1 inch in depth, and not quite $\frac{3}{4}$ inch in width, and is fitted with rackwork so that half a dozen slides can be taken. The box goes comfortably in the waistcoat pocket. Films of blood, sputum, etc. can be carried without risk of their being damaged by dirt or moisture.
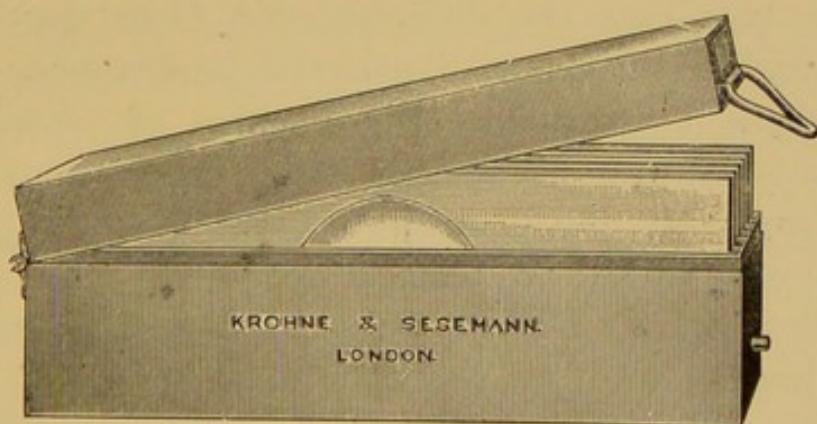
FIG. 2.

The Acid=Fast Bacteria :

THEIR RESEMBLANCE TO AND DIFFERENTIATIONS
FROM THE TUBERCLE BACILLUS.

INTRODUCTION.

The discovery within the last few years of micro-organisms resembling the tubercle bacillus is of great interest to the bacteriologist and of vital importance to the physician.

A few years ago every acid-fast organism was either the bacillus of leprosy or of tuberculosis. 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 tubercle in its general morphology, and particularly in its acid-fast character.

The power of resisting decolorisation by mineral acids after being deeply stained was supposed to render the recognition of Koch's bacillus by means of the microscope easy and certain to all. The bacillus of leprosy is so infrequently encountered—at least, in this country—that the differentiation from the bacillus of tuberculosis was not of material importance.

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

Moeller, Petri, Rabinowitsch, Lubarsch, and many others have found several apparently different species of bacilli, which not only closely resemble the tubercle bacillus in form and size, but which retain the stain when, after being coloured according to Ziel-Neelsen's

method, they are subjected to the action of acids and alcohol. They further resemble the true bacillus of tuberculosis in that when inoculated into animals they produce nodular or tubercular-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, in the secretions and excretions of many herbivora, and also in normal and pathological secretions in man.

They have been seen in tonsillar exudation, in caries of the teeth, in the sputum from cases of non-tubercular abscess and gangrene of the lung.

Moeller has found them in grass, hay, pollen, dust of the stable, and in the dung of cattle.

Rabinowitsch found acid-fast bacilli in 28·7% of samples of butter examined in Berlin and Philadelphia.

It would have been somewhat surprising if only the bacilli of tubercle 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 bacillus."

Lubarsch, Moeller, Bulloch, and many others have stated that the microscopical examination is now not sufficient for the diagnosis of tubercle. The last-named observer[10] states that "it would now appear that there are quite a number of bacilli which are as acid-fast to acids and alcohol as any genuine tubercle bacillus, and it must be acknowledged that this discovery has given a rude shake to the belief that the microscopical 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, with the finest

lens, indistinguishable from the genuine organism of tuberculosis. I have carefully examined most of these bacteria in pure cultures, and in their natural condition, after subjecting them to exactly the same method of staining, with a magnification of 2,250 diameter—*i.e.*, by means of an apo-chromatic $\frac{1}{12}$ objective, with No. 18 compensation eyepiece. As a result of such an examination, I can find some difference between the *majority* of the pseudo and genuine tubercle bacilli; but here and there are organisms which, as far as I can see, it is impossible to distinguish.

If this difficulty exists when we are dealing with a more or less pure culture, and can compare them 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 appearance in the same culture or in the same specimen of sputum.

My attention was drawn to these tubercle-like organisms by my having found acid-fast bacilli resembling tubercle in the urine of three 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 disease. In one case this was confirmed by two leading English pathologists, but was disproved by a Continental bacteriologist. In the second case inoculation experiments with guinea-pigs gave absolutely negative results. In the third case, the after-history of the patient pointed distinctly to the non-tubercular nature of the disease.

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 decolour-ising agents—*e.g.*, acids, alcohol, acid alcohol, etc.—and as a result have found what I consider 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 follow-ing acid-fast organisms :

> The bacillus of tuberculosis, and its modifications.
> The tubercle bacillus of birds and cold-blood animals.
> The bacillus of leprosy.
> The bacillus of smegma.
> The bacillus of Timothy grass (Moeller).
> The grass ii. bacillus of Moeller.
> The mist or dung bacillus (Moeller).
> The butter bacillus of Petri-Rabinowitsch and Korn.

In addition, some forms of acid-fast streptothrix—viz., that of Leishman and Birt, of Eppinger and Nocard.

I will then describe the morphological differences which exist between these; and, lastly, the results of my inquiry 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 a quarter to a half the diameter of a red blood corpuscle (1·5 to 4 μ). Although the length varies, the 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 first stage of corkscrew structure" (Bulloch). He also, in his original description, described them as containing oval spores, two to six being present in a single bacillus. (Plate I., Fig. 1).

Cornet and Mayer describe them as graceful rods with somewhat rounded ends, measuring about 2 to 4 μ long, i.e., from a quarter, to three quarters the diameter of a red blood corpuscle, and about 0·3 to 0·5 μ broad.

According to Lehmann and Neumann,[2] they are 1·5 to 4 μ long, and only 0·4 thick; according to Macé,[3] 1·5 to 3·5 μ long, having a general 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.

The bacilli occur sometimes singly, often in clusters, and, especially in urine, in the form of tufts. They are usually found in the mucus background of the preparation, but not infrequently are inside the cells, which may be crammed full of them.

In stained preparations from old cultures, and in sputum, particularly that from a cavity, a number of uncoloured spots or vacuoles, as many as 5 or 6, may be seen, giving the bacillus an appearance somewhat like a short chain of cocci. These, at first regarded as spores, are now generally considered as vacuoles, and represent a degenerative change, or even an accumulation of reserve material within the bacilli.

In addition to the unstained vacuoles alternating with the stained parts of the bacilli, roundish granules somewhat larger than the body of the bacillus may occasionally be seen. There may be one, usually two, rarely three of these in an organism, and they are generally found near the extremities. They take the stain much more deeply than the body of the bacillus, and are usually of a brown or a black-red colour. According to Coppen Jones, these granules resist the decolourising effect of nitric acid longer than the rest of the organism.

They are found in some specimens of sputum from a cavity, and to a less extent in old cultures. They are not spores, but what their exact nature is seems undetermined.

These characters as regards shape and size are by no means constant, either in the organism met with in cultures or in sputum. Sometimes they are very short, according to Macé little longer than broad; at other times they are very much larger, and may be swollen or clubbed at the extremities.

Metchnikoff, Nocard, Roux, Babes, Klein, and others have found giant forms, filament and thread forms, and true branching forms. These more or less exceptional forms are chiefly met with in old cultivations, but also occasionally in sputum. Moeller has seen true branching forms 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. 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 bacilli. These stained very deeply with the fuchsin, and were considerably wider than the body of the bacillus in which they lay. I have seen these 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 vegetation 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 a rod which reproduces 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, on 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 branched rods with clubbed ends, were met with.

Friedrich[7] and Nösske obtained these clubbed actino-mycotic 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 growth by inoculation with the pseudo-tubercle bacillus, notably Moeller's Timothy bacillus, grass bacillus ii., and mist bacillus.

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

The *cultivation* of the tubercle 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 fresh strains of

tubercle 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 tubercle bacillus has begun its first division" (Bulloch[11].)

Pawlowsky found that glycerinated potatoes in sealed tubes formed a good medium, and this has since been largely used. Glycerine potato-juice, agar or bouillon, were recommended by Lubinski.

Hesse found that 0·5 per cent. of "Nahrstoff Heyden" (a soluble albumin) with 3 per cent. glycerine gave rapid growths from tubercular sputum in one to three days, and, according to Bulloch and Frankel, this is one of the best media.

The tubercle bacilli grow best ·at temperature of the human body, viz., 37° C., the minimal and maximal limit being 29° to 42° C ; therefore probably it does not thrive well outside the human body.

Czaplewski[18] found that he could get a growing culture of tubercle bacillus to show signs of an increased growth at the temperature of the room. I have also seen cultivations made by my friend, Dr. Arthur Ransome, grow at the temperature of the laboratory.

Moeller, by passing the tubercle bacilli through a blind worm, succeeded in growing them at 20° C.

The characteristic *staining reaction* of the tubercle bacillus depends on the fact that it is not easily stained, but when stained resists decolorisation by mineral and organic acids. Koch originally succeeded by the addition of an alkali in staining it, but this method is not used now. Generally speaking, the

tubercle bacillus is best stained by using a warm or hot solution of a strong basic aniline dye—*e.g.*, gentian violet or fuchsin—combined with a mordant—*e.g.*, aniline water or carbolic acid—the usual combination being aniline gentian violet (Ehrlich-Koch) or more generally carbolic fuchsin (Ziehl-Neelsen). The various modifications of staining will be described later.

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

It has been proved that watery solutions of a basic dye, if used hot, can stain the bacillus, but the colour is only partially fast. Bulloch states that when stained in this manner the bacilli are decolourised within one and a half 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 decolorisation, so that parts of the bacillus—oval parts, situated mostly at the poles—retain the stain and are sharply demarcated. These egg-like bodies, Ehrlich says, may retain the stain under the influence of sodium sulphite for eight to ten 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 permeable for aniline, alkalies, etc., but impermeable for acids.

Koch[1] himself 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 hairs, the capsule of the coccidium oviforme, and the ova of tape worms (Bulloch). According to Czaplewski, certain keratinized cells and the nuclei of mast cells resist partial decolorisation (Macé[3]).

Cornet aud Meyer mention also the following :— some epithelial structures, certain spores (according to Lichtheim, Neisser and Bienstock), crystals of fatty acids (Gaffky, Petri), mast cell granules (Orth), and lanoline (Gottstein), but remark that none of these are likely to be confused with the tubercle bacilli.

The acid-fast power of the tubercle bacillus was thought to be due to the presence of fat in the bacillus, and Dorset recommended the use of Sudan iii., a fat stain, as a means for staining the bacillus.

Aronson,[12] in 1898, definitely showed that the *saurefestigkeit* 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 they still retain their power of resisting acid ; but if hydrochloric acid is added to the mixture, they are no longer, according to Bulloch, acid-fast.

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

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

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

McLeod and Bulloch have isolated this waxlike substance both from the tubercle and Timothy grass bacillus.

Bulloch,[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; it is difficult to grow, is not adapted for a saprophytic existence, and requires high temperature for growth. Under all conditions it is acid-fast, due to the presence of a waxlike body.

Klein[19] and Marmorek[20] have found that quite young tubercle bacilli are not resistant to acids and alcohol. The latter thinks that this is due to the fact that young tubercle bacilli 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 decolorising them. Klein suggests that the chemical substances which are ordinarily present in tubercle bacilli, rendering them resistant to acids, are absent from very young bacilli.

MODIFIED TUBERCLE BACILLI: AVIAN TUBERCLE.

In the tubercular disease of birds, bacilli are found which correspond in their morphological characters and staining reactions with those in mammals, but differ in cultures and experimental inoculations. These organisms were first distinguished from the tubercle bacillus by Maffucci.[13]

Fischel[14] says that both mammalian and avian tubercle bacilli are one and the same kind as regards nutritive media. He succeeded in getting the tubercle bacilli of mammals acclimatised 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 he was able, by injecting avian tubercle bacilli, to induce a general tuberculosis, but cultures obtained from this animal were not identical with those of avian tubercle.

Avian tubercle bacilli must be regarded as only a form of the mammalian tubercle which has become accustomed to the higher temperature of birds, but which is also occasionally pathogenic for other animals. It requires a higher temperature for its growth 42° C., or a minimum and maximum of 35° to 45°. Cultures of bird tubercle have generally a damper and smoother growth on artificial media, but exceptions to this are not infrequent.

Lubarsch[9] recognised three forms of growth:

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

2. Dry, wrinkled skins which are not easily rubbed apart.

3. Cultures which 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. Krase[21] found that cultures which at first resembled closely those of tubercle bacilli became damp and soft later, whilst Lubarsch has frequently noticed 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 found that when injected into guinea-pigs, after considerable time had elapsed the bacilli could be found in the infected tissues arranged in a radial manner, as is the case with the 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 (Moeller).

Dubard and Bataillon[15] cultivated an organism resembling the tubercle 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 tubercle bacillus acclimatised to cold-blooded animals by inoculating and feeding fishes and frogs with cultures of human and avian tubercle, and from the organs of such fish the bacillus piscicola was obtained.

Lubarsch found that the tubercle bacilli of fishes produced pathologically about the same effect as the tubercle bacillus from a blind worm, and only once did he find bacilli arranged in a radial manner. He also was able to modify the tubercle bacillus of mammals by passage through frogs, so that they grew at a temperature of 28° to 30° C.

Moeller[16] isolated from the spleen of a blind worm, which had a year previously been injected with human tubercular sputum, cultivations of tubercle which flourished at 20° C. Cultures of these, Lubarsch states, instead of being dry and crumbly, were damp with shiny white surface. They do not grow at 28° to 37° C., but grow best at a temperature of 22°. Morphologically, they are indistinguishable from tubercle bacilli, and cultures, according to Moeller, resemble those of bird tuberculosis.

They cannot be inoculated into rabbits.

The Leprosy Bacillus.

Discovered by Armauer Hansen in 1877, and more fully described later by Neisser, the bacillus of leprosy has long been known as an acid-fast bacillus resembling the tubercle bacillus. They are usually thin rods, measuring about $5\,\mu$ to $6\,\mu$ long, and $0.3\,\mu$ broad, generally slightly curved. When stained they show either a uniform or beaded appearance, darkly stained parts alternating with unstained points. There is no evidence that spores can be seen in their interior, and they are non-motile. They often appear tapered at one or both extremities, and sometimes club-shaped swellings are seen. They are more constant in size and are generally a little shorter than the tubercle bacillus.

They stain by the Koch-Ehrlich and Ziehl-Neelsen's method, and also by Gram. They resist decolorising, but not quite to the same extent as the tubercle bacillus (Muir and Ritchie, Woodhead), and they take the aniline basic dyes more readily, and can be more readily stained by the watery solution of these dyes, than Koch's bacillus.

Macé,[3] however, states that they resist acids better than the tubercle bacillus, and, according to Babes, dilute nitric acid does not decolourise them after an hour, whilst at the end of this time the tubercle bacilli are always decolourised.

Lehmann and Neumann[2] state that the leprosy bacillus cannot be certainly differentiated from the tubercle bacillus, although it is said that the leprosy 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, while the

tubercle bacillus is not. On the contrary, alkaline methylene-blue is said to stain the tubercle quicker than the lepra bacillus. " Still, all authors are now agreed that the staining reaction cannot help much in the differential diagnosis, any more than the form of the bacilli, from which it follows that the separation of leprous and tuberculous affections in the cadaver appears often impossible, since at least it is made by different persons." According to Hansen and Looff, tuberculosis is responsible for 40% of the deaths in lepers, and obviously this fact further increases the difficulty of distinguishing them. Lehmann and Neumann give the following differential chart, taken from an article in the *Centralbl. f. Bakter.* by Spiegel,[23] from Unna's laboratory.

	LEPROSY.	TUBERCULOSIS.
Number of Bacilli :	Exceedingly abundant in all organs and secretions	Very much less numerous
Arrangement of the Bacilli :	In heaps like a cigar in form	More as individuals or in irregular bunches
Form :	Rod shaped, straight, and plump	Thready, curved, and fine
Angles :	Sharp	Roundish
Appearance of Granules :	Coarse	Fine
Arrangement of Granules :	Widely separated	Close together

These differences are naturally never so typical as here appears.

Hansen says the leprosy bacilli retain the stain as

the tubercle bacilli do; they are, however, more easily stained than the latter, but the difference is so slight that it is not practicable for diagnostic purposes.

Method of examination for the bacillus of Leprosy. —For clinical purposes it will be sufficient to pick out one of the cutaneous nodules, if these be present, and squeeze out a little fluid, from which films are made and stained as for tubercle.

Manson (*Lancet*, August 23, 1884) gives an excellent method of demonstrating, for diagnostic purposes, the presence of the leprosy bacillus in the infiltrated leprous patches.

"A leper tubercle or infiltrated patch is selected, and the whole or part of it included in the jaws of an ordinary thin-bladed pile clamp. The tightening of the clamp has the effect of driving out all the blood from the included tissues, and the tubercle, from being dirty red or purple, becomes like yellow wax. The hold of the clamp is maintained at a degree of tightness sufficient to keep up a state of anæmia, and at the same time the centre of the included mass is pricked with a needle or sharp knife. From the puncture a droplet of perfectly clear fluid exudes, and is to be transferred to one or more coverglasses, each coverglass being smeared with rather a thick layer of leper juice. The coverglasses are then dried, stained, washed, and mounted in the ordinary way. Under the microscope, slides so prepared show bacilli in prodigious numbers, both free and in dense bundles, packing the leper cells."

Muir & Ritchie[24] say that the presence of large numbers of bacilli situated within the cells and giving the staining reactions of leprous bacilli is conclusive,

and consider that in most cases there is really no difficulty in distinguishing the two organisms.

Macé 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.

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

Hansen could not obtain a pure culture of the lepra bacillus, but in 1887 Bordoni-Uffredrusi obtained a culture on glycerine serum from the marrow of a leper, but was unable to preserve it. Babes 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 were very much like the tubercle bacillus. The growth obtained by the two last-named investigators differs, according to Moeller, from the true cause of leprosy by its uncertain acid-resisting power, and is classed by Czaplewski as intermediate between the diphtheria and tubercle group of organisms. Inoculations, however, failed to produce leprous changes.

E. van Houtum[25] has quite recently announced that he has succeeded in cultivating the bacillus of leprosy. He used a mixture consisting of $\frac{1}{3}$ beef broth and $\frac{2}{3}$ fish broth. In this broth the organisms grew rapidly, and at the end of twenty-four hours, at 36° C., there is a general turbidity.

The Smegma Bacillus.

In 1885 Tavel and Alvarez,[26] whilst investigating the so-called bacillus of syphilis discovered in 1884 by Lustgarten,[27] found in the normal preputial smegma an acid-resisting bacillus—the bacillus of smegma.

This organism is found especially in the smegma of the prepuce, in the secretions of the outer skin, particularly where a collection of epithelium occurs, as in the anal and vulvar 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 reactions, 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 Neumann states that it is the general opinion that Lustgarten's positive findings in gummas were to be explained by a mixed infection with tuberculosis.

The smegma bacillus, as described by Tavel and Alvarez, is extremely like the bacillus of tubercle in shape, size, and staining reaction. It can be stained by the same method, but is said to be less resistant to alcohol. The discoverers found that inoculation experiments on animals gave negative results.

Matterstock,[28] about the same time—1885—found an acid-fast bacillus in smegma which corresponded in morphological characteristics and staining reactions with that described by Tavel and Alvarez, but he also was unable to obtain a pure culture.

Laser[29] and Czaplewski[30] in 1897 independently

obtained cultures of micro-organisms resisting de-
colorisation by acids, resembling the diphtheria bacillus,
which they stated were identical with the bacillus of
smegma. Laser obtained his cultures from syphilitic
disease, Czaplewski from gonorrhœal pus.

Fraenkel[31] denied that these diphtheria-like bacilli
were identical with those described by Tavel, Alvarez,
and Matterstock, and he only considers those organ-
isms smegma which resembled the tubercle bacilli.

Fraenkel found that Laser and Czaplewski's organisms
did not resemble the tubercle bacilli; that they were
more like pseudo-diphtheria bacilli, and that in later
generations they lost their acid-resisting power.

Moeller,[17] after examining this culture, agrees with
Fraenkel, and states that he was unable to get patho-
genic effects in guinea-pigs with the diphtheria-like
bacilli cultivated from smegma, or with secretions
containing the real smegma bacilli in abundance.

Neufeld[32] has also cultivated from smegma these
acid-resisting diphtheria-like bacilli, which were in-
distinguishable from Czaplewski's, and which possessed
a moderate acid-resisting power. He had repeatedly
noticed acid-resisting bacilli 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 non-acid-fast forms,
and succeeded by cultivation in obtaining a great
increase in the tubercle-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 bacilli. Neufeld there-
fore came to the conclusion that in smegma two types
of acid-fast bacilli were present—those like diphtheria
and those like tubercle bacilli.

Moeller[17] 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 healthy person, after producing a blister with emp. cantharidis, tubercle-like, acid-fast bacilli were present among the epithelial cells. These were present in very small numbers, but, by placing the serum and . pieces of skin in the incubator, he found that these were greatly increased in number after forty-eight hours. After three to four 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.

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

Morphologically, the bacilli show great variation. In young cultures they appear as slender, sometimes slightly bent rods, and are often delusively 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 bacilli, alterations in form are seen —e.g., threads, rods with unstained vacuoles, with club-like swellings, with deeply stained granules and coccothrix forms. The bacilli show no movement.

Their staining reaction is very like that of the tubercle bacillus. They are absolutely acid- and alcohol-fast (Moeller), independently of the medium on which they are grown. They are not decolourised by exposure to 3 per cent. HCl alcohol for twelve minutes, and the bacilli are stained in the cold by dilute carbol-fuchsin. Pure cultures react to the differential stains of Bunge and Trantenroth and Pappenheim

exactly as the tubercle bacillus. The acid and alcohol resistance is not diminished in later generations. Thus Moeller found its reaction the same in the twenty-fifth generation as in the original culture.

Cultivations.—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 three 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, and after twenty-four hours a layer of colonies is visible. At the temperature of the room the growth is slower. They grow on all the usual media. On glycerine-agar, at 37° C., colonies appear as small dull gray-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 gray-white, dull colonies are seen.

In milk they grow rapidly and luxuriantly, and milk forms an especially 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 three to four days. If the tube be shaken, tiny fragments fall to the bottom.

Inoculation experiments are negative. Moeller inoculated smegma contained in various secretions of

the skin in rabbits, into hens and doves and guinea-pigs, and has never obtained any pathogenic results. The smegma-bacillus, he says, differs in this way from other acid-fast bacteria.

Johannes Barranikow,[33] however, states that he has obtained very different results, and as a result of his experiments, draws the following conclusions:

1. Inoculation of preputial smegma from the body of a non-tubercular adult, and from a healthy living child, produced in guinea-pigs local and universal appearance of disease, just like that produced by the inoculation of sputum containing tubercle bacillus.

2. That smegma of various domestic animals (preputial, mammary, etc.) gave the same results by inoculation—viz., exclusively general tuberculosis.

Both these statements, Barranikow says, are in direct contra-distinction to the views and investigations of Moeller.

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

4. It is necessary to investigate the whole life-history of the organism.

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

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

7. The acid-fastness and non-resistance to acids are only transitory conditions of the microbe.

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

The *differential diagnosis* of smegma bacillus from true tubercle bacillus is of the greatest importance. This is especially so of the genito-urinary tract, and acid-fast bacilli found in this region could not be declared tubercle bacilli.

Moeller, in doubtful cases, differentiates all 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 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 tubercle, but pseudo-tubercle. The true tubercle bacillus requires a temperature of 37° C. 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 when the sputum is mixed with certain nutritive 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, forty-eight hours, whilst in the pseudo-tubercle bacillus a persistent further proliferation takes place at 30° C." (Moeller.)[35]

TIMOTHY GRASS BACILLUS (MOELLER).

Moeller[34] found this bacillus in grasses used for fodder, and as they were first discovered on the Timothy grass—*Phleum pratense*—he named them Timothy grass 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 twelve to twenty-four hours obtained a fairly pure growth of the hay bacillus.

Moeller kindly brought to my notice Lubarsch's experiments. He obtained his grass from two different places and made infusions in sterilized water in a flask, and kept them at a temperature of 37° C. At the expiration of eighteen hours he found, mixed with the hay bacillus, numerous acid- and alcohol-fast bacilli, which were, according to Lubarsch, easily distinguished from the tubercle bacillus by their greater thickness and length. I found that mere infusion of the inflorescent part of the grass usually gave negative results, but when the whole of the grass was cut into small pieces, and infused for twelve to twenty-four hours at 37° C. a few acid-fast bacilli answering to Moeller's description were found. I am not at all sure that they can be obtained from all Timothy grass, but have certainly found them in other grasses, notably the *Alopecurus pratense*, *Bromus erectus*, and the common foa. I found, as Lubarsch states, that after forty-eight hours the acid-fast bacilli are almost completely outgrown by the hay bacilli. Lubarsch managed to get a pure culture by making his infusion with very little water, and examining hourly till such time when the Timothy bacillus were

very numerous (in from nine to thirteen hours), and then by means of agar plates isolated the bacilli.

Moeller, in a private communication, states that he has also obtained the bacilli from pollen.

The Timothy, or grass i. bacillus, takes the form of little rods, which are microscopically very like, and, according to Moeller, often indistinguishable from, the tubercle bacillus. Lubarsch, however, states that they are fairly easily distinguished by their greater thickness and length. Like the tubercle bacillus, this bacillus often contains deeply-stained granules, and also in some cases oval unstained patches. It divides into branches, and sometimes club-shaped swellings are found at one end. True branchings are seldom seen, and then only in dilute bouillon cultures and on Fraenkel's albumin-free medium (Lubarsch). They are not motile.

The bacilli grow on all the usual nutritive media, best at incubation temperature, indifferently at room temperature. When grown at 37° C., distinct patches of colonies are seen, and these sooner or later become coloured. On glycerine-agar plates 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 glycerine-agar are of a brighter orange red colour, moist at first, but after a time become wrinkled. In bouillon there is a variable condition. Sometimes a thin pellicle is formed over the surface, and the fluid may remain clear or become turbid, but frequently a precipitate of a yellowish colour occurs.

It grows well on potato, the colonies appearing as yellowish, moist elevations.

Moeller[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 it, and like the tubercle it becomes slower in its growth.

This pseudo-tubercle bacillus is alcohol- and acid-fast, and behaves in the same way as the tubercle bacillus does with the ordinary staining methods. Lubarsch[9] thinks that the Timothy bacillus in the tissues of animals is not quite as resistant against decolourising methods as the tubercle bacillus, and in this way its resisting-power is like the bacillus of leprosy. 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 the Timothy bacillus, mixed with sterilized butter, is injected into guinea-pigs, general 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, giant cells, epithelial cells, and caseation, a condition very like genuine tuberculosis, is produced. As Lubarsch says, "there can be no doubt whatever that it is quite impossible to distinguish for certainty by histo-logical or micro-parasitic examination between Timothy fungus tubercles and true tubercles: the distinction can only be brought about by cultures. In all animals injected with Timothy bacillus a negative reaction to tuberculin was obtained" (Moeller[35]).

Lubarsch's[9] animal experiments were briefly as fol-lows: An eight-day-old glycerine-agar culture of the Timothy bacillus was injected into the kidney of a guinea-pig. At the end of thirteen days a small piece

of the kidney at the point of injection was excised, and a small yellowish mass the size of a lentil was found, apparently of a caseous material. This, microscopically, showed typical tubercular appearance—viz., masses of large epithelioid cells, amongst which are Langerhans' giant cells, and around these a proliferation of uninucleated round cells.

In the centre of this nodule the bacilli were arranged in typical radial mass, like actinomycosis, with club forms.

On the thirty-first day after the injection a piece of nodule which had formed on the kidney was excised, and it was seen that caseation had increased and the genuine Langerhans' giant cells were more numerous. The radial arrangement of the bacilli was still present, and although not more numerous than on the thirteenth day, they were larger and the clubs longer and thicker.

GRASS II. BACILLUS (MOELLER).

Moeller[37] described this as "a new acid- and alcohol-fast bacillus of the tubercle bacillus group, which shows genuine branching."

This organism he found in the pollen on the stable floor, in fodder grass, and in the dust of grasses generally, and was able to isolate it on gelatine plates. It takes the form of little rods, sometimes like cocci in fluid media, and morphologically and in its staining reactions it is very similar to the tubercle bacillus, but is generally somewhat thicker. When grown upon moist media it appears as little rods, which are longer and thicker than the tubercle bacillus (Lubarsch). Like the other pseudo-tubercle bacillus associated with Moeller's name, it is absolutely acid- and alcohol-fast, especially in fresh cultivations.

In older cultures, especially those made on solid media, and kept at 37° C. for four or five days,

thread and branching forms are found, and these stain a pale-red with Ziehl-Neelsen's stain, having lost somewhat their power of resisting acids.

On agar the growth is very luxuriant, and when kept at 37° C. on glycerine-agar streak, after two days, small drop-like colonies, which later run into one another, are seen. The culture is then somewhat delicate, and after a few days the colonies become yellowish in colour, and in the water of condensation little skin-like flakes are seen. Lubarsch, in his account, says, that, unlike the radial fungi generally, its growth on agar is soft and pulpy.

On potato at 37° C., the growth is luxuriant, and thick grayish-white colonies are seen.

In milk the growth is very rapid, and there is an acid re-action after two to three days.

In bouillon, after three to four days at the temperature of the room, there is turbidity, and on shaking a thread-like sediment falls. A whitish-gray pellicle forms on the surface, and tends to grow up the side of the tube.

On a streak culture on gelatine at 20° C., after four to five days thick grayish-white colonies appear. No liquefaction of the gelatine occurs.

Moeller[37] has stained these organisms by most of the tubercle bacilli stains—*e.g.*, Fraenkel's, Ehrlich's, Gabbet's, Czaplewski's, and Ziehl-Neelsen's—and finds that the bacilli in their young state resist decolourisation by mineral acids and alcohol just as the tubercle bacillus. They are stained by Gram's method. They possess amœboid movement only in their young condition.

In size the bacilli vary very much. Most of them are about 1 to 5 μ long, and 0.2 to 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. Similar to what has been observed in the tubercle bacillus, they often arrange themselves in the shape of a Y. Long branched and unbranched threads are found in the margins of the colonies, especially those grown on glycerine-agar for three to four days at 37° C., and sometimes fragment forms and coccothrix, particularly in milk cultures. The threads often have deeply-stained granules, which are much broader than the body of the bacillus. Swellings at one or both ends are sometimes seen. The branched forms are usually made up of a long thread, from which other thread-like branches or short clubbed swellings start off at right angles, unlike the acute-angled branching of the cladothrix. The fine branches often divide again.

Moeller states that he has seen similar branching of the tubercle bacillus in sputum, and Kral and Dubard have found the same in the tubercle bacilli of cold-blooded animals. Zopf, of Halle, considers, with Moeller, that these, like some forms of the tubercle bacillus, show true branching, and not the false branching of the cladothrix, which would tend to show that these, like the tubercle bacilli, 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 ii. bacillus, as found in cultures, is rather less resistant to acids and alcohol than the tubercle bacillus or some of the pseudo-tubercle bacilli.

Inoculation into Animals.—According to Moeller, guinea-pigs, intraperitoneally injected with a pure culture of grass ii. bacilli, died at the end of four to six

a bacillus that it could not be easily overlooked, especially in films which have been well decolourised in the acid.

Lubarsch, while recognising its close resemblance to the Timothy bacillus, is able to draw certain points of distinction between these closely-allied species.

On agar both organisms grow in a similar manner. Bouillon never becomes diffusely turbid by the dung bacillus, and colonies are rarely found at the bottom of the tube, as is generally the case with the Timothy bacillus.

Upon Gasperini's medium the Timothy bacillus grows luxuriantly, the mist bacillus sparingly. The mist bacillus less frequently forms true branching. The further slight differences he found in the effect 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 uni-nucleated elements like epithelioid cells were not found so frequently, but true radial arrangement of the bacilli was much earlier.

In one case typical radial masses with clubs were formed as early as the sixth day. In order to determine more fully the occurrence of radial or actinomycotic forms of the mist bacillus in animals, Lubarsch injected directly into the kidney of guinea-pigs a culture of the mist bacillus. In one at the end of six days, and in another on the eighth day, radial masses of bacilli with clubs were found at the point of inoculation, lying in the giant cells and partly surrounded by leucocytes. These differed from those resulting from the injection of the Timothy bacillus 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.

Butter Bacillus (Petri-Rabinowitsch).

Owing to the fact that the tubercle bacilli were found in such appalling frequency in butter and milk, investigations were started by Petri[38] and Rabinowitsch[39] to determine whether these organisms were the genuine tubercle bacillus.   Both these observers found in butter and milk a bacillus which, although it is acid- and alcohol-fast, is not the true tubercle bacillus.   As the organisms isolated by these observers separately are so much alike, they are generally spoken of as the Petri-Rabinowitsch's butter 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 true tubercle bacillus.   It resists acids and alcohol exactly as the true tubercle bacillus, but in sections, according to Moeller, it is not as resistant to acids.

It grows well at room temperature, and at incubation temperature there are visible signs of growth after twenty-four hours.   When grown on the ordinary media the colonies are distinctly different from those of tubercle, but closely resemble the latter on Proskau's albumin-free medium and in bouillon.

According to Lehmann and Neumann,[3] when grown on glycerine-agar plates the growth has the appearance of wrinkled, irregularly dentate scales, of a transparent grayish-white colour.   Later they become opaque, of a brown-gray colour, and irregularly marked.   Streak cultures on glycerine-agar appear as wrinkled, dry formation, very like that of true tubercle bacillus, and

easily mistaken for it in the very young condition. Later the growth becomes somewhat orange to copper-red colour (Rabinowitsch),

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

Grown on potato, 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 scarcely at all glistening.

Lubarsch[9] emphatically contradicts Lydia Rabinowitsch's statement that the butter bacillus is identical with the Timothy and dung bacillus of Moeller. He states that they differ in morphology, in cultures, and in animal experiments.

It is longer and thinner, and, especially when grown in thin bouillon, shows genuine branching and clubs more frequently than the other pseudo-tubercle bacillus. On agar, he says, the butter bacillus remains much longer colourless, and does not become a yellow colour till the eighth day, and then does not possess the intense colour that the colonies of the Timothy and mist bacillus do.

Unlike Timothy bacillus, a wrinkled pellicle is formed on the surface of bouillon after forty-eight hours, and a precipitate soon occurs, leaving the bouillon translucent, whilst early clouding of bouillon is characteristic of the Timothy bacillus. Animal experiments produce less changes than Moeller's bacilli.

When injected with butter into guinea-pigs, Moeller found changes which macro- and micro-scopically might easily be mistaken for genuine tubercle. When injected in pure cultures alone, less effects were produced than when butter was injected with it. Lubarsch

found in the infected areas the radial arrangement of the bacilli, with club formation. Langerhans' giant cells, nests of epithelioid cells, and typical tuberculosis caseation are never found in the foci of the disease (Rabinowitsch).

Korn[40] has isolated another acid-fast bacillus from butter, which he has termed the *B. freburgensis*. This, he claims, differs from the Petri-Rabinowitsch's butter bacillus morphologically and culturally, but especially in its action on animals.

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

Korn gives the following as characteristic :

" 1. Stains by Ziehl-Neelsen's method especially well, and is little influenced by acids.

" 2. Uniform, not interrupted, growth in gelatine stab.

" 3. The surface of the agar culture is depressed in the centre, the peripheral zone being elevated.

" 4. Upon bouillon it produces a disagreeable, 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 intraperitoneal injection of 0·5 cc. of a suspension. The animal dies in from four to forty days, and presents massive nodules in all the abdominal organs " (Lehmann and Neumann[2]).

Moeller has also obtained a pure culture of a bacillus from milk, similar to the tubercle bacillus, which does not differ materially from the grass ii. bacillus.

"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" (Moeller[35]).

Acid=resisting Bacilli found in Human Secretions and Excretions.

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.

A. 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, in the sputum of a case which post-mortem examination proved was a non-tubercular bronchiectasis associated with a small gangrenous abscess of the lung.

Lydia Rabinowitsch also found them under similar conditions, and proved by pure cultures that they were probably a variety of the butter bacillus.

Marzinowski found acid-fast bacilli in the crypts of the tonsil, in five out of twelve cases which he examined. He also found them in a case of bronchitis, Karlinski in the healthy and diseased nasal cavity.

Cima found, in the pus of eight cases of middle ear suppuration in children, no less than 6 in which bacilli resisting decolourisation by Gabbet's method were present.

Aoyama and Miyamoto found acid-fast streptothrix in a non-tubercular abscess of the lung, and Dittrich in a purulent ovarian cyst.

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

Moeller[16] has, he says, frequently found pseudo-tubercle bacilli in the mucus from the nose, pharynx, coating on the tongue, sordes on the teeth, and secretions on the tonsils. During an attack of bronchitis he found in his own expectoration small grayish nodules which contained acid-fast bacilli in great numbers. By further investigation he proved that these were not genuine tubercle bacilli, and, as he says, this was corroborated by the fact that his lungs were absolutely healthy three years after.

He mentions further 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 there was a considerable increase in these acid-resisting organisms. On re-inoculating fresh medium, 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 bacilli, and the unreliability of mere microscopic diagnosis, mentions the following illustrative cases :

1. Case in which rods stained well by 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 a guinea-pig ; no tuberculosis developed, and the further history of the case negatived the diagnosis of phthisis.

2. 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. Post-mortem examination of the patient : no signs of pulmonary tuberculosis. The bronchial secretion post-mortem also contained these bacilli.

3. Secretions from a bronchial cavity contained pseudo-tubercle bacilli, which produced negative results when injected into guinea-pigs. No evidence found at post-mortem examination of tubercular disease.

4. 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. After history was opposed to that of tuberculosis.

5. In the cystic swelling on the forearm of a medical man who had had symptoms of tuberculosis as a child, he found acid-fast bacilli morphologically identical with the tubercle bacillus, but cultivations and inoculations into guinea-pigs were negative.

He asserts that he proved conclusively that the second, fourth and fifth case were not tuberculous, in spite of the presence of acid-resisting bacilli. In the other cases the bacilli may have been dead or attenuated. Against this assumption he points out

that guinea-pigs are so sensitive to tuberculosis that that they may be killed by the injection of fluids in which no tubercle bacilli can be demonstrated microscopically.

"It follows that in doubtful cases, which include all in which an examination of a pathological liquid is likely to be made, a bacterioscopic 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 minority of cases, the only remaining reliable test is injection of the fluid into susceptible animals."

PATHOGENITY AND DIFFERENTIATION OF THE PSEUDO-TUBERCLE BACILLI GENERALLY.

Moeller[16] states that all the pseudo-tubercle bacteria resistant to acids, except the bacilli of smegma, have this in common, that they produce a tuberculous disease in the usual animals operated upon—the true tubercle bacilii always, but the pseudo-tubercle bacilli only in a limited number of cases and under certain conditions. The pseudo-tubercle bacilli are especially virulent if injected with butter into animals, and when intraperitoneally given always cause peritonitis with extensive induration. If genuine tubercle bacillus, to-gether with butter, is injected intraperitoneally into animals, typical tuberculosis does not result, but indurative peritonitis, just as is the case with pseudo-tubercle bacillus and butter. He inoculated six calves with human tubercle bacilli, grass bacilli, and pseudo-perlsucht bacilli, with and without butter. The calves before operation did not react to the tuberculin test ;

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the butter in all cases was sterilized. As a result 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 the tubercle bacillus hominis alone produces the same perlsucht-like appearance in calves that the pseudo-tubercle bacillus alone does ; and that the tubercle bacillus hominis with butter causes the same disease appearance as the pseudo-tubercle bacillus with butter does.

Lubarsch,[9] and, later, Mayer and Holscher, have proved, by numerous experiments on guinea-pigs and rabbits, that the pseudo-tubercle bacillus can produce, even without butter, a condition closely resembling tubercle. The resemblance of pseudo with real tuberculosis is so close that even Moeller says a practised eye may easily be deceived ; but on fuller examination considerable differences will be found.

Differentiation.—Flugge[21] has called attention to a method of differential diagnosis, by means of inoculation. If true tubercle-bacilli be injected into the anterior chamber of the eye 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 the true tubercle bacillus always results in a general infection, whilst pseudo-tubercle bacillus does not produce such a result—only an abscess at the point of inoculation occurs. As to the differences in their transference, true tuberculosis can be carried by means of the infected organs from one animal to another, but this cannot be done with pseudo-tubercle ; animals can only be inoculated with a pure culture of the bacilli taken from the infected organs.

The clinical effects produced by inoculation also differ. Animals inoculated with true tubercle bacilli show, soon after the injection, more or less signs of illness. They lie about as if feeble, are feverish, appetite disappears, and in spite of attention as to food, etc., they become thinner and weaker, and die. Post-mortem examination shows general tuberculosis. Animals inoculated with pseudo-tubercle bacilli soon show, after the injection, somewhat similar signs, but these quickly pass off, and they run about, eat well, increase in weight, and appear quite healthy ; and one is surprised to find, after killing them, distinct pathological changes.

Further differences are seen histologically. True tubercles are mostly of a solid, proliferative nature, and later go on to caseation ; pseudo-tubercles are of a more inflammatory type, with a tendency to abscess formation.

Most important, however, is the fact that the tubercle bacilli, when inoculated, increase in number, whilst the pseudo-tubercle bacilli appear only as a foreign body, and there is no increase in their number, except when they are present in large numbers, or carried by the blood or lymph stream. When the pseudo-tubercle bacillus is 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 bacillus this is of no importance.

It has not been proved that the pseudo-tubercle bacillus is pathogenic for man, and as a result of general observation, this is probably not the case. In no case—not even when these bacilli have been found in disease conditions—can they be proved to have any etiological connection with any disease in man.

The close resemblence between the pseudo and genuine tubercle bacillus in morphology and staining reaction is only an apparent relationship; the fundamental fact is that the 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 tubercle bacillus, and in cultures they may be alike, and the tubercle bacillus may be accustomed to a more rapid growth at a lower temperature, yet they will always remain distinct organisms. They cannot be transferred 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 (Moeller).

An Acid-fast Streptothrix Pathogenic to Man and Animals.

Majors Birt and Leishman,[42] R.A.M.C., report a case in which they found streptothrix which were acid-fast. Briefly, the case was that of a private who, whilst beleagured at Ladysmith, contracted fever and dysentery. On his arrival at Netley he was found to be suffering from right pleural effusion and great enlargement of the liver. On examining the reddish, muco-purulent expectoration, acid-fast rods resembling the tubercle bacillus were found, and at the same time a few thin segmented, branched filaments, also acid-fast, were seen, and thought to be actinomycotic forms of the bacillus of tubercle.

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

and in sections of the infected areas in the lung, acid-fast organisms closely resembling 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 branches at right angles. In length they stretched almost across the field of the microscope ($^1/_{12}$ oil immersion). Their width, which was fairly uniform, measured about 0·5 μ, and showed no signs of clubs or spore formation. The threads stain well with all the basic dyes, and retain Gram's stain. After staining with Ziehl-Neelsen and decolourising in 25 per cent. sulphuric acid and alcohol, they remain deeply stained.

Cultures.—The growth is very slight, if any, at the room temperature. On gelatine plates, after five to seven days at 22° C., white circular colonies formed, which resembled balls of cotton-wool, snow white by reflected, and slightly yellow in the centre by transmitted, light. No liquefaction of the medium occurred after three weeks' incubation.

On *agar* growth is rapid. After 36 to 48 hours snow-white, dry, powdery growth appears, which later becomes pale pink. In nutrient broth, after 24 to 36 hours at 37° C., 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 below.

Potato 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 third to fourth day.

The streptothrix appears in cultures in two forms, a fine branching of mycelial threads, and a so-called streptococci 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. When young the threads and arthrospores retain the fuchsin intensely, but gradually the threads lose this 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 observers noticed that bodies deeply stained black by Weigert's method were seen in the threads, but nothing like clubs or involution forms were found.

When inoculated intraperitoneally into guinea-pigs death occurred in from five to six weeks, and large collections of caseous matter were found in the peritoneal cavity. When inoculated under the skin similar caseous matter formed, and in this, in both cases, the organisms were found chiefly in the form of threads.

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

Eppinger, in 1890, 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, termed it *Streptothrix Eppingeri*, and Flugge speaks of it as a streptothrix. Lachner Sandoval, in 1898, pointed out that it cannot be classified with the oospora, and called it *Actinomyces asteroides*.

MacCallum[44] found this organism in the pus of a peritoneal abscess. He describes it as a branched

filamentous organism with no evidence of septum formation. The branching is true, but not dichotomous. It stains readily with the ordinary aniline dyes, 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 Neisser's method very distinct blackish-blue granules were seen in its interior. 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.

Lubarsch found that by inoculating a culture of this organism into guinea-pigs, true radial growths, like the actinomyces, were produced.

Neither MacCullum nor Macé mention its staining reaction with Ziehl-Neelsen, but Berestnew states that they stain by this method.

Birt and Leishman admit that this streptothrix of Eppinger is not unlike the one which they have found morphologically, but differs from it by growing to some extent anaerobically, by the characteristic growth in bouillon—dense layers of felt-work, which sink to the bottom, and are renewed again and again—and by the orange tint which the cultures take on.

Another acid-fast organism, the *Streptothrix bovis*, was found by Nocard[45] in 1898, in the disease known as "farcin du bœuf." This is apparently a rare affection of oxen occurring in the island of Gaudeloup, characterized by a suppurative inflammation of the lymphatic glands and vessels. Nocard states that it does not stain well with the ordinary aniline dyes. According to Berestnew, it stains by Ziehl-Neelsen's method, but Nocard says it does not.

I shall endeavour shortly to give the results of my enquiry into the morphological differences, and the degree of resistance to acids, alcohol, etc., of the tubercle bacillus and the pseudo-tubercle bacilli.

THE MORPHOLOGICAL CHARACTERS OF THE TUBERCLE AND PSEUDO-TUBERCLE BACILLI COMPARED.

1. *Tubercle Bacilli,*—Stained of a brilliant red, of slender but variable breadth, usually somewhat bent, and often showing a beaded appearance (Plate i. Fig. 1). In cultures, especially the older, the internal structures, the alternation of clear and darkly stained areas, more pronounced.

2. *Smegma Bacilli in Cultures,*—Stained of a darker red, owing to their being slightly affected by the after-staining with methylene blue, more opaque and denser. In size very variable, but generally shorter and much thicker than the tubercle bacillus. There is a tendency to the wedge- or spindle-shaped form, like young diphtheria bacilli. Cocci forms are more frequent. There is much less sign of internal structures.

3. *Smegma Bacilli in Smegma,*—Bacilli of very variable size, but generally shorter than the typical tubercle bacillus. The majority are in the form of opaque rods, with more or less rounded, not pointed ends. They do not present the appearance of diphtheria bacilli quite as much as those 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 tubercle bacillus is generally absent. Their width varies very much, but usually they are

distinctly thicker and coarser than the tubercle bacillus, but very many of them cannot be distinguished from the latter. Their colour, as I notice in all the pseudo-tubercle bacilli, is darker and denser, and less transparent than Koch's bacillus. A very few show a large, centrally-placed, deeply-stained granule like that I have occasionally seen in the tubercle bacillus in sputum. Sometimes very thick forms—two or three times the thickness of the ordinary bacilli—are seen. The majority are somewhat thicker at one end than the other, and most are slightly bent.

4. *Timothy Bacilli*,—Stained of a darker red. Size and shape very variable; generally speaking, thicker and coarser than the tubercle bacillus. The sides are often not parallel. The bacilli are thicker in the centre, and tail off towards their extremities. Very little sign of internal structure is seen. They look dense. A few ghost-like forms are met with in cultures, and these stain faintly, are not well defined, and are often the longest present.

5. *Grass ii. Bacilli*,—Stained an opaque dark red; the extremities are squarer and the sides more parallel than any of the other pseudo-tubercle bacilli. Although very variable in size, they are longer than the other pseudo-, and distinctly plumper than, the tubercle-bacilli. Short cocci forms are often seen. The grass ii. bacilli were the only pseudo-tubercle bacilli in which I could find granules stained by Neisser's method (*vide infra*).

6. *The Mist or Dung Bacillus* is least like the tubercle bacillus of all. It is the shortest and thickest. It shows no internal structure, and has usually both extremities pointed, and is much more spindle-shaped —to my mind rather like a grain of wheat. In older

cultures it may appear much longer and more like the other pseudo-tubercle bacill.

7. *The Streptothrix of Birt and Leishman.*—Thanks to Dr. Leishman, I was able to study this acid-fast organism. At first sight, film preparations made from old cultures have the general appearance of the mist bacilli—*i.e.*, short, thick rods—but on further examination the organism is seen to consist largely of cocci, very short bacilli, long rods and long threads or filaments, mixed up together.

Determination of the Degree of Resistance to Acids, Alcohol, etc., of the Acid=fast Bacilli.

I have made the following experiments to determine to what extent the acid-fast bacilli—viz., tubercle, smegma, Timothy, grass ii., mist or dung bacilli, and the streptothrix of Birt and Leishman—resist decolourisation by acids, alcohol, etc.

I have used for this purpose sputum containing numerous tubercle bacilli, a pure culture of human tubercle bacilli on potatoes about nine months old, and pure cultures on agar of the following organisms obtained from Kral's laboratory : Smegma bacillus, Timothy bacillus, grass ii. bacillus, and mist bacillus. Dr. Leishman has kindly sent me a pure culture of the acid-fast streptothrix (Birt and Leishman).

I have also experimented on impure growths of these organisms, which I have obtained—viz., smegma bacillus from preputial smegma, Timothy bacillus from an infusion of *Phleum pratense*, grass ii. from an infusion of hay-fodder and the mist bacillus from cow-dung.

The following is the *method* I have adopted :

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

2. 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 (never allowing actual boiling or drying of the solution to occur) for a few seconds, and then allowed to stain. The whole staining process lasted seven minutes. At the end of this time the film containing the fuchsin was well washed in cold water from the tap, and allowed to dry in the air. It was then subjected to the decolourising reagent. The Ziehl-Neelsen's solution was that ordinarily used, having the following formula: Basic fuchsin, 1 ; absolute alcohol, 10 ; solution of carbolic acid in water (1 in 20), 100 parts.

In order that the films should be subjected to plenty of the decolouriser, I first poured this over the film, and allowed it to rest there a minute, and then the reagent—acid or alcohol—was poured off, and the film placed in a wide-mouthed bottle containing the decolourising agent. By this means evaporation was prevented.

In order that each organism should be subjected to exactly the same time in fixing, staining, and de-colourising, in addition to the ordinary films, I made eight such films on the same slide, and by this means was assured that each was exposed to precisely the same conditions in all stages of the experiments.

I. RESISTANCE TO 25 PER CENT. SULPHURIC ACID.

It has been very generally understood, and repeatedly stated in text-books, that after a time the tubercle bacillus is very easily decolourised by acids, and that in the ordinary method of staining sputum, care should be taken that the preparation was not subjected too long to the action of the acid. Macé[3] for example, states : "When decolourising by acids 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 ten to fifteen minutes' duration. The use of organic acids as decolourising agents easily prevents this mistake."

Abbott and Gildersleve,[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, e.g., 3 per cent. HCl in alcohol, 5 per cent. H_2SO_4 in water—but are almost at once decolourised by 25 to 30 per cent. nitric acid in water."

The same observers (in *New York Med. Record*, May 10, 1902, p. 748), state that "they had found that practically the whole group of pseudo-tubercle bacilli were almost immediately decolourised by the usual acid mixtures employed for decolourisation in connection with the examination for tubercle bacilli. This was true not only of the old nitric acid decolouriser, but when the now popular 5 per cent. (*sic*, probably means 25 per cent.) H_2SO_4 was employed."

Further, they say that "they had found it so easy to differentiate these acid-resisting bacilli by the old and well-known methods that they had not extended their observation to other and new preparations."

The results of the following experiments clearly show how very much my observations differed from these statements :

SULPHURIC ACID, 25 PER CENT.

Films stained as above were subjected to this acid.

1. *At the end of twenty minutes—*
   - (*a*) Tubercle bacillus in sputum : densely stained red.
   - (*b*) Tubercle bacillus in culture : densely stained.
   - (*c*) Smegma bacillus in culture : well stained.
   - (*d*) Timothy bacillus in culture : well stained.
   - (*e*) Grass ii. bacillus in culture : well stained.
   - (*f*) Mist bacillus in culture : well stained.

2. *At the end of one hour—*
   All the above six forms well stained.

3. *At the end of two and a half hours—*
   All the above well stained.

4. *At the end of four hours—*
   - (*a*) Tubercle bacillus in sputum and in cultures : well stained.
   - (*b*) Smegma bacillus in culture : many bacilli still red.
   - (*c*) Timothy bacillus in culture : most of the bacilli still red.
   - (*d*) Grass ii. bacillus in culture : decolourised.
   - (*e*) Dung or mist bacillus in culture : quite red.

5. *At the end of seven hours—*
 (*a*) Tubercle bacillus in cultures and sputum : distinctly red.
 (*b*) Smegma bacillus in cultures : many still red.
 (*c*) Timothy bacillus in cultures : about half stained red, .
 (*d*) Grass ii. bacillus : completely decolourised.
 (*e*) Mist bacillus in cultures : mostly blue, but large patches of red.

6. *At the end of nine hours—*
 (*a*) Tubercle bacillus in cultures and sputum : beautifully red.
 (*b*) Smegma bacillus in cultures : decolourised.
 (*c*) Timothy bacillus in cultures : many, especially the spherical forms, red.
 (*d*) Grass ii. bacillus : decolourised.
 (*e*) Mist bacillus : some exceedingly well stained red.

7. *At the end of sixteen hours—*
 Tubercle bacillus in sputum and cultures : well stained.

 Smegma bacillus, Timothy bacillus, grass ii. bacillus, and mist bacillus : all decolourised.

8. *At the end of twenty-four hours—*
 All the pseudo-tubercle bacilli : decolourised.
 Tubercle bacillus in cultures and in sputum : still red.

9. *At the end of forty-hours—*
 Tubercle bacillus in cultures and sputum : still red.

10. *At the end of seventy-two hours—*
 Tubercle bacillus in cultures : exceedingly well stained brilliant red.
 Tubercle bacillus in sputum : brilliant red, and all seem stained.

Conclusions.

1. That all resist the action of 25 per cent. H_2SO_4 for two and a half hours.

2. That at the end of sixteen hours all the pseudo-bacilli are decolourised; the tubercle bacilli not decolourised.

3. That the grass ii. bacillus is decolourised the earliest.

4. That of the pseudo-tubercle bacilli, the mist and Timothy bacilli resist the longest.

5. That tubercle bacilli in sputum and in cultures were exceedingly well stained after seventy-two hours.

II. Nitric Acid, 33 per Cent.

1. *After five minutes' immersion—*
   Tubercle bacillus in sputum and cultures, smegma bacillus in cultures, Timothy, grass ii., and mist bacilli: all stained.

2. *After thirty minutes—*
   (*a*) Tubercle bacillus in sputum and cultures: distinctly stained.
   (*b*) Smegma bacillus in cultures: partially decolourised and stained bluish by the methylene-blue.
   (*c*) Timothy, grass ii., and mist bacilli: stained distinctly red.

3. *At the end of fifty minutes—*
   (*a*) Tubercle bacillus in sputum and cultures: distinctly red.
   (*b*) Smegma bacillus in cultures: many decolourised, but many also distinctly red.
   (*c*) Timothy bacillus: about half red and rest blue.

(*d*) Grass ii. bacillus: most of them distinctly red.

(*e*) Mist bacillus: well stained.

4. *At the end of two and a half hours—*

(*a*) Sputum—tubercle bacillus: fainter red.

(*b*) Culture—tubercle bacillus: distinctly red.

(*c*) Smegma bacillus in cultures: many decolourised, many red.

(*d*) Timothy bacillus: mostly decolourised, a few red.

(*e*) Grass ii. bacillus: apparently completely decolourised.

(*f*) Mist bacillus: very distinct bright-red colour.

5. *At the end of five hours—*

(*a*) Tubercle bacillus in sputum: apparently many not stained at all, some faintly, others fairly red.

(*b*) Tubercle bacillus in cultures: distinctly red, but paler.

(*c*) Smegma bacillus: many quite red, but most stained blue.

(*d*) Timothy bacillus: mostly blue, a few red.

(*e*) Grass ii. : completely decolourised and stained blue.

(*f*) Mist bacillus: mostly red, even in the thinnest parts of film.

6. *At the end of twenty-four hours—*

All the pseudo- and tubercle bacilli: decolourised.

III. ALCOHOL.

It is generally stated in nearly all text-books that the smegma bacillus is easily distinguished from the tubercle bacillus by the fact that the former will not resist decolourisation by alcohol, but the latter will.

Even Kruse in Flugge's "Micro-organisms" makes this statement.

Nencki and Podezaski,[48] even as recently as this year (1902), say that the smegma bacillus, although acid-proof, has not much resistance against alcohol : "If the preparations, after being stained in the usual way with Ziehl-Neelsen's solution and treated with acid, are subjected to alcohol, the smegma bacillus will lose its red colour and appear blue when the preparation is after-stained with methylene blue."

Nikitin[49] states that :

" 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-Neelsen's 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 per cent. Methylated.

Films stained in the manner described are placed first in sulphuric acid, 25 per cent., for seven minutes ; then placed, after washing, in alcohol.

1. *After five hours in alcohol—*

    (*a*) Tubercle bacillus in sputum and cultures : well stained red.

    (*b*) Smegma bacillus in cultures : most, but not all, decolourised, some distinctly red.

    (*c*) Timothy bacillus : many quite red, many blue.

(*d*) Grass ii. bacillus : many, especially in the thinner parts of the film, decolourised, others not.

(*e*) Mist bacillus : not decolourised at all.

2. *After nine hours in alcohol*—

(*a*) Tubercle bacillus in sputum and culture : still stained red, but they appear darker from the blue of the after-stain.

(*b*) Smegma bacillus in cultures : most are decolourised, but many are deep red.

(*c*) Timothy bacillus : many stained red.

(*d*) Grass ii. bacillus : majority are decolourised and stained blue.

(*e*) Mist bacillus : majority stained deeply red, some blue.

3. *After twenty-four hours in alcohol*—

(*a*) Tubercle bacillus in sputum and cultures : still red, but they take on a blue tint and appear a dark colour from after-stain.

(*b*) Smegma bacillus in cultures : decolourised.

(*c*) Timothy bacillus : reddish blue—distinctly a dark-red colour.

(*d*) Grass ii. bacillus : almost entirely decolourised and stained blue, a few bacilli red.

(*e*) Mist bacillus : red colour.

4. *After twenty-eight hours in alcohol*—

(*a*) Tubercle bacillus in sputum and cultures : red, with a tinge of blue, clear and distinct.

(*b*) Smegma bacillus in cultures : decolourised.

(*c*) Timothy bacillus : still reddish blue.

(*d*) Grass ii. bacillus : completely decolourised, blue.

(*e*) Mist bacillus : stained red and blue, mostly red.

IV. Acid-Alcohol.

A. Honsell's Method.—This method is described in several of the text-books as a reliable one for differentiating the smegma from the tubercle bacillus. Hewlett[50] describes the method as follows : "After staining in warm carbol-fuchsin, the specimen is washed and dried. It is then to be immersed in acid-alcohol (3 per cent. HCl) for ten minutes, washed in water, and counter-stained for a few seconds in saturated alcoholic solution of methylene-blue, washed, dried, and mounted. Smegma bacillus is decolourised." Lehmann and Neumann give the method much as this, with the exception that they counter-stain in alcoholic solution of methylene-blue diluted one-half with water.

The following are the results I obtained with this method :

1. *After ten minutes in 3 per cent HCl alcohol*—
   - (*a*) Tubercle bacillus in sputum and cultures : stained red.
   - (*b*) Smegma bacillus in cultures : practically decolourised ; a few, however, are stained red.
   - (*c*) Timothy bacillus : well stained red.
   - (*d*) Grass ii. bacillus : part decolourised ; part, especially thicker portions, distinctly red.
   - (*e*) Mist bacillus : decolourised.

2. *After one and a half hours*—

   Tubercle bacillus in cultures and sputum : stained faintly.

   Timothy bacillus : stained only in thick parts of film.

   All the other pseudo-bacilli decolourised.

3. *After fifteen and a half hours—*
     Tubercle bacillus in sputum: stained faintly. In cultures: the tubercle bacilli are quite evident. All other forms decolourised.

4. *After seventeen hours—*
     Tubercle bacillus in sputum: still stained, but faintly.

5. *After twenty-four hours—*
     Tubercle bacillus in sputum: practically all decolourised.

ACID-ALCOHOL 3 per cent. HCl, and counter-stained with aqueous solution of methylene-blue.

1. *At the end of one hour—*
     (*a*) Tubercle bacillus in sputum and cultures: stained red.
     (*b*) Smegma bacillus in cultures: partially decolourised
     (*c*) Timothy bacillus: very faintly stained.
     (*d*) Grass ii. bacillus: stained red.
     (*e*) Mist bacillus: stained red.

2. *At the end of three hours—*
     (*a*) Sputum—tubercle bacillus: red, but fainter.
     (*b*) Tubercle bacillus in cultures: mostly stained slightly, but fainter.
     (*c*) Smegma bacillus in cultures: many well stained.
     (*d*) Timothy bacillus: completely decolourised.
     (*e*) Grass ii. bacillus: mostly stained, less than half decolourised.
     (*f*) Mist bacillus: stained.

3. *At the end of seventeen and a half hours—*
     (*a*) Tubercle bacillus in sputum: red, getting fainter. In cultures tubercle bacilli are faint red.
     (*b*) All the pseudo-bacilli decolourised.

4. *After twenty-six hours—*
- (*a*) Tubercle bacilli in sputum : a few distinctly red, most are decolourised.
- (*b*) Tubercle bacilli in cultures : in most cases quite decolourised, a few red bacilli faintly stained may, however, be seen.

V. BUNGE AND TRANTEROTH'S METHOD.

This is another method given for distinguishing the smegma bacillus from the tubercle bacilllus.

Lehmann and Neumann and Hewlett describe it as follows :

1. Cover-glass preparations are immersed in absolute alcohol for three hours.

2. They are then placed in 5 per cent. chromic acid for fifteen minutes.

3. Stained in warm carbol-fuchsin for two to three minutes.

4. Decolourise in 25 per cent. sulphuric acid for two to three minutes.

5. Counter-stain in concentrated alcoholic solution of methylene blue for at least five minutes.

The smegma bacillus is said to be decolourised.

I found this method useless for differentiating any of the pseudo-tubercle bacilli, including the smegma bacillus, from the bacillus of tuberculosis.

Even after seven minutes in 25 per cent. H_2SO_4 the following results were obtained :
- (*a*) Tubercle bacillus in sputum and cultures : stained red.
- (*b*) Smegma bacillus in cultures : all reddish-blue or bluish-red.
- (*c*) Timothy bacillus : a few quite red.
- (*d*) Grass ii. bacillus : bluish-red.
- (*e*) Mist bacillus : some quite red.

VI.   Fraenkel's and Pappenheim's Method.

This is also another method advised for distinguishing the smegma bacillus from the tubercle bacillus.

Simons (in *Clinical Diagnosis*) describes the process as follows: "After staining in Ziehl-Neelsen's solution, immerse from three to five times in Fraenkel's and Pappenheim's solution, care being taken to let the fluid drain off slowly after each immersion." Pappenheim's solution consists of "1 part corallin (rosolic acid) 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 found that, when used in the above manner, all the pseudo - tubercle bacilli — including the smegma bacillus in cultures and in smegma—remained red, and not blue, as is supposed to be the case.

I therefore tried to find a time limit at which all pseudo-tubercle bacilli would be decolourised, and the extreme limit to which the bacillus of tubercle would resist.   The following are my results :

1. *After seven and a half minutes in Pappenheim's solution—*

> (*a*) Tubercle bacillus in sputum and cultures : well stained red.
>
> (*b*) Smegma bacillus in cultures : partially decolourised ; is mostly stained blue ; a few distinctly red.
>
> (*c*) Timothy bacillus : half stained blue, half red.
>
> (*d*) Grass ii. bacillus : all stained blue.
>
> (*e*) Mist bacillus : nearly all stained red.

1. *After thirty minutes in Pappenheim's solution—*

> (*a*) Tubercle bacillus in cultures and sputum : well stained red.

(b) All the pseudo-tubercle bacilli, except a few Timothy and mist bacilli, decolourised and stained blue.

3. *After one hour in Pappenheim's solution—*
Tubercle bacillus in sputum and cultures : well-stained red.

All the pseudo-tubercle bacilli, except a very few Timothy bacilli, decolourised and stained blue.

4. *After one and three-quarter hours, three and a half, thirteen, and seventeen hours respectively—*
Tubercle bacillus in sputum and in cultures : well-stained red.

5. *After twenty-six hours—*
The tubercle bacillus in sputum and cultures still distinctly red, although a little darker in tint from the methylene-blue.

All pseudo-tubercle bacilli decolourised and stained blue.

6. *After fifty-two hours—*
(a) Tubercle bacillus in sputum : most of them distinct red, some bluish-red, but all easily distinguished.
(b) Tubercle bacillus in cultures : most of them red, some bluish-red, but distinct.

I further tried the effect of staining films with Ziehl-Neelsen's solution for one hour in the cold, without any heat, and then placed such stained films in Pappenheim's solution for half an hour. Result : all the pseudo-tubercle bacilli decolourised, and only a few of the tubercle bacilli in sputum and cultures were stained red.

PAPPENHEIM'S ROSOLIC SOLUTION *without methylene-blue,* but after-staining with weak watery solution of methylene-blue. The following were the results :

After staining films in the usual way with carbol-fuchsin, and placing in Pappenheim's solution, without methylene-blue for *one and a half hours*, all the pseudo‑tubercle bacilli decolourised ; the tubercle bacillus well-stained red.

After twenty-four hours.—Tubercle bacilli in sputum and cultures stained red, but somewhat faintly.

VII. Gram's Method.

(*a*) *Tubercle Bacilli in Cultures.*—The effect is not at all unlike that of Neisser's method. Small deeply stained dots are seen at the end of most of the bacilli. Many of the shorter bacilli have only terminal dots, but in the longer forms there is often a central spot, sometimes two, whilst in the longest variety there is quite a number of dots of equal or unequal size. The body of the bacillus is only very faintly stained by the gentian violet.

(*b*) *Smegma bacillus in cultures* are stained by Gram, the bacilli appearing like young diphtheria bacilli—no granules.

(*c*) *Timothy, grass ii.*, and *mist bacillus* all stain by Gram, but show no granules.

VIII. Neisser's Granule Stain.

The only organism showing granules is the grass ii. bacillus. In cultures of these bacilli I found that the majority contained deeply stained bluish‑black granules at the extremities, sometimes also one placed centrally. The body of the bacilli does not, however, take on the stain. I have adopted a modification of Neisser's method, previously described in the *British Medical Journal*, 1899.

IX. Effect of Watery Solution of Aniline Dyes in the Cold.

When films are stained with a concentrated aqueous solution of methylene-blue (Grubler's pure) for *ten minutes* at the temperature of the room, the following results are obtained :

(*a*) Tubercle bacillus in sputum and cultures not stained.

(*b*) The pseudo-tubercle bacilli—viz., smegma, Timothy grass ii., and mist bacillus—are all well stained. This bears out the statement that the tubercle bacillus is not easily stained by aqueous solutions of aniline dyes.

X. Ziehl-Neelsen's Solution in the Cold.

Films were stained for *twenty minutes* with Ziehl-Neelsen's solution, at the temperature of the room, without heat, and were decolourised in 25 per cent. H_2SO_4 for the following period :

1. *After two minutes in 25 per cent. H_2SO_4—*
   All the pseudo-tubercle bacilli stained.
   The tubercle bacilli in sputum and cultures : few stained, and these faintly.

2. *At the end of ten minutes—*
   Tubercle bacilli in sputum : few seen. In cultures the tubercle bacilli, some distinct, some faint.
   Smegma bacillus : reddish blue.
   Timothy bacillus : many distinctly red.
   Grass ii. bacillus : mostly blue.
   Mist bacillus : most are faintly stained red.

3. *At the end of forty minutes*—

    Sputum : no tubercle bacilli were seen.

    In cultures : many quite red and distinct ; some very faint, others apparently decolourised.

    Smegma and grass ii. bacilli : completely decolourised.

    Timothy bacillus : most a faint red, some distinctly blue, a few distinctly red.

    Mist bacillus : reddish pink, not distinct.

This bears out the statement that the tubercle bacilli are not so easily stained in the cold as the other pseudo-tubercle bacilli.

ZIEHL-NEELSEN'S SOLUTION IN THE COLD.

Films stained for *one hour* at the temperature of the room, and then decolourised, for the time stated, in 25 per cent. H_2SO_4.

1. *After fifteen minutes in 25 per cent. H_2SO_4*—

    Sputum : very few tubercle bacilli found, and these clear red.

    Cultures : tubercle bacillus well-stained.

    Smegma bacillus in cultures : purplish colour, some quite red.

    Timothy bacillus : mostly red, some blue.

    Grass ii. bacillus : nearly all decolourised, a very few red.

    Mist bacillus : quite red.

2. *After three quarters of an hour in 25 per cent. H_2SO_4*—

    Sputum : very few bacilli found.

    Cultures : tubercle bacilli, most of them well-stained.

    Smegma bacillus in cultures : more or less decolourised, purplish.

Timothy bacillus : many red.

Grass ii. bacillus : completely decolourised.

Mist bacillus : quite red.

3. *After three and a half hours—*

Only the tubercle bacilli in cultures are visible. Tubercle bacilli in sputum : none seen. All the pseudo-tubercle bacilli decolourised.

XI. FILMS PREVIOUSLY TREATED WITH ALKALIES.

Macé[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 fat. By treating the preparation for ten minutes with 'une lessive de soude additionnée de 5 p. 100 d'alcool,' one removes the fat, and at the same time its power of resisting decolourisation. Under the same conditions the bacillus of tuberculosis retains its stain after the action of the acid."

In order to ascertain whether this statement was of any practical value, I tried the two following experiments :

I. FILMS PREVIOUSLY TREATED WITH LIQUOR POTASSÆ FOR TEN MINUTES, washed, dried, and then placed in ether for five minutes, and subsequently in alcohol for a few minutes. These, when stained with hot Ziehl-Neelsen's solution in the usual way, had lost some of their resistance to acids.

II. I further subjected films, air-dried and fixed by moderate heat, to the action of the following solution, for half an hour :

Aqueous solution of caustic soda (1 : 12)...40 parts.

Alcohol, 90 per cent. methylated............10 ,,

They were then washed, dried, and stained in the

usual way with hot Ziehl-Neelsen's solution, and, after again washing and drying, placed in 25 per cent. H_2SO_4 for the time stated.

1. *At the end of half an hour in 25 per cent. H_2SO_4—*
   Tubercle bacillus in sputum and cultures : well-stained red.
   Smegma bacillus in cultures : nearly all red.
   Smegma bacillus in smegma : well-stained.
   Timothy bacillus : some still red.
   Grass ii. bacillus and mist bacillus : very few red.

2. *At the end of one and a half hours—*
   Tubercle bacillus in sputum and cultures : well-stained red.
   Smegma bacillus in cultures : many stained red.
   Smegma bacillus in smegma : many stained red.
   Timothy bacillus : many of the smaller forms still red.
   Grass ii. and mist bacillus : a few cocci forms still red.

3. *At the end of two and a half hours—*
   Tubercle bacillus in cultures : stained red.
   Smegma bacillus in cultures : many stained red.
   Smegma bacillus in smegma : many distinctly red.
   Timothy and mist bacilli : a very few red, chiefly cocci forms.
   Grass ii. bacillus : almost decolourised.

The Smegma Bacillus as seen in Smegma.

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

For this purpose I have used entirely human preputial smegma, and in my experience the three following conditions seem to be met with :

1. The number of bacilli of smegma found in preputial smegma varies very greatly. That in some they exist in enormous masses, in others they are very scanty. I have found that in the smegma of adults they were in some cases very infrequent, whilst in that of a young girl aged about seven they were exceedingly numerous.

2. That the bacilli of smegma, as met with in smegma, behave very differently in different specimens with regard to their resisting powers against acids and alcohol.

In some smegma, especially a specimen obtained (without antiseptics) from a circumcision on an adult male, the bacilli were almost immediately decolourised by H_2SO_4; in fact, it was, I found, in these particular cases, impossible to obtain good specimens. After the action of 25 per cent. sulphuric acid for a second or so, very few bacilli stained red were seen. This, of course, might have been because they were not present in the film, but on preparing another film from the same specimen, and using about $12\frac{1}{2}$ per cent. sulphuric acid for a few seconds, I found them very numerous. By examining such a preparation mounted

in water, I was able to remove the coverglass, and, having logged the position of a group of bacilli by means of the mechanical stage, I tried the effect of pouring on 25 per cent. H_2SO_4 and off immediately. On again examining the patch, I found that very many of the red bacilli were completely decolourised.

This feeble resistance to acids may be due to— (a) the presence of much fatty matter in the film, preventing the carbol-fuchsin staining well, and therefore the organisms, being slightly stained, are easily decolourised; or it may be that (b) the smegma bacilli under different conditions, which are at present unknown, behave very differently to the action of decolourising agents.

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

3. The bacillus of smegma in preputial smegma taken from a young girl I found to be very much more acid-resisting than the last named. It resisted decolourisation by acids and alcohol as long as the smegma bacillus in cultures—*i.e.*, practically as long as any of the pseudo-tubercle bacilli with which I am familiar.

The following are the results of my experiments with this smegma:

I. Films of preputial smegma, fixed by heat and stained with hot Ziehl-Neelsen's solution exactly in the manner previously described, were then immersed in 25 per cent. H_2SO_4 for the time stated, and counter-stained with a weak aqueous solution of methylene-blue;

1. At the end of fifteen minutes, thirty minutes, forty minutes, seventy minutes respectively, in 25 per cent. H_2SO_4, the smegma bacilli were well stained red.

2. At the end of one and a half hours many of the bacilli well stained.

3. After two and a half hours, many of the bacilli decolourised, but some are distinctly stained red.

4. After four hours' immersion in 25 per cent. H_2SO_4, a few bacilli are still clearly stained red, although most are decolourised.

5. At the end of eight hours, still a few bacilli, especially in the thicker part of the film, are red.

6. At the end of nine and a half hours, very few bacilli still red, chiefly the spherical forms.

7. At the end of thirteen hours, all smegma bacilli decolourised.

II. Films of preputial smegma, dried in the air, and without other fixation, placed for fifteen minutes *in soda-alcohol* (formula given above), then stained in usual way with carbol-fuchsin, and placed in 25 per cent. H_2SO_4:

At the end of four hours, many bacilli exceedingly well stained.

At the end of eight hours, a few bacilli stained.

At the end of thirteen hours, all decolourised.

Comparing the power of resistance which films fixed by heat and those previously treated with soda in alcohol possess, I should say that the latter, far from rendering them easily decolourisable, rather tends to prolong the time necessary for complete decolourisation. This is exactly in opposition to the statement of Macé.

III. Smegma bacilli in smegma, after staining in the usual way with carbol-fuchsin, were all decolourised at

the end of half an hour in *acid-alcohol* (3 *per cent. HCl*).

IV. Films of smegma, placed for fifteen minutes in 25 per cent, H_2SO_4, and then in *alcohol* (90 per cent. methylated) :

> At the end of half an hour, smegma bacilli not decolourised.
>
> At the end of one hour, many decolourised.
>
> At the end of two hours, all decolourised.

V. In *Pappenheim's Solution :*

> At the end of half an hour, smegma bacilli not decolourised.
>
> At the end of one and a half hours, many bacilli decolourised ; not all.
>
> At the end of three hours, all decolourised, except a few spherical forms.
>
> At the end of four hours, all decolourised.

VI. In *Nitric Acid* ($33^1/_3$ *per cent.*) :

> After forty minutes, nearly all smegma bacilli decolourised, but the background still red, and a few red bacilli seen.
>
> At the end of sixty minutes, practically all decolourised.

STREPTOTHRIX OF BIRT AND LEISHMAN.

Resistance to acids and alcohol varies somewhat with the age of the cultures, but the average of my results was as follows :

I. *Sulphuric Acid* 25 *per cent. :*

> At the end of half an hour, cocci forms not decolourised.
>
> At the end of one and a half, two and a half, and six and a half hours, nearly all the cocci forms are stained.

II. *Pappenheim's Solution :*

At the end of half an hour, cocci forms not decolourised.

At the end of one and a half hours, nearly all cocci forms decolourised.

At the end of three hours, all decolourised.

III. *Acid Alcohol (3 per cent. HCl):*

At the end of half an hour, all cocci forms decolourised.

I can confirm the observations of Birt and Leishman that the streptothrix *threads* in cultures after a time lose their acid-fast character, and, as they say, in old cultures they are very easily decolourised by the ordinary methods. The so-called cocci or rod-forms of this streptothrix are the most likely to be mistaken for tubercle bacilli.

CONCLUSIONS.

1. The tubercle bacillus is not the only acid- and alcohol-resisting bacillus. In addition to the bacillus of leprosy, there are several other acid-fast bacteria, amongst which are smegma, Timothy, grass ii., mist or dung, butter bacilli, and one or two species of streptothrix.

2. The acid-resisting property depends upon the presence of a waxy substance in the body of the bacillus. This is found in the pseudo-tubercle as well as the tubercle bacillus.

3. The tubercle bacillus can withstand prolonged action of acids and alcohol, much longer than is generally stated.

4. The acid-resisting pseudo-tubercle bacilli resemble the genuine tubercle bacilli, not only in their acid-fast power, but also in their size, shape, and form, and also

in the fact that in many cases they tend to produce in animals nodular diseases, in which giant cells, etc., are found. They are all probably members of the actino-mycotic group.

5. Although the tubercle and pseudo-tubercle bacilli are all acid-fast, yet they are not equally resistant. The tubercle bacillus withstands the decolourising action of acids and alcohol considerably longer than any other pseudo-bacilli with which I have experimented.

6. The pseudo and genuine tubercle bacilli cannot safely for diagnostic purposes be distinguished by their mere morphological characters.

7. 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 decolourized, and how long the tubercle bacilli, on the other hand, can safely resist the action of the decolouriser, and then to ascertain whether the results so obtained are constant.

8. Among other results, my experiments show that the tubercle bacilli can resist 25 per cent. sulphuric acid for at least, in some cases, seventy-two hours, but that all the pseudo-tubercle bacilli are decolourised at the end of sixteen hours, many before that time.

That tubercle bacilli in sputum and in cultures resist the decolourising action of Pappenheim's solution well for at least fifty-two hours, whilst all the pseudo-tubercle bacilli are decolourised at the end of four hours.

From these experiments, I would suggest the *following methods for the distinction of the tubercle bacilli from all other acid-fast pseudo-tubercle organisms :*

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

    ii. Dry in the air, and fix by passing through flame of a spirit-lamp in the usual way.

    iii. Whilst still warm, pour on filtered carbol-fuchsin, allowing it to spread over the slide, and allow it to remain for half a minute. 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 seven minutes.

    iv. At the end of this time, thoroughly wash in running water, and decolourise in either of the following solutions :

        (*a*) *In Pappenheim's Solution.* Place the film in a wide-mouthed bottle containing this solution for at least four hours, and not longer than twelve hours. Wash in water, dry, and mount in oil or balsam. The tubercle bacilli are the only organisms stained red.

        (*b*) *In Pappenheim's Solution alone—i.e., without the addition* of methylene blue. This is, in my opinion, the best method. The time limit is the same as in (*a*). Wash in water, and after-stain for a minute or so in a weak aqueous solution of methylene-blue. This method has the advantage that the tubercle bacilli

remain brilliantly red, and not the dark bluish red as in (*a*).

(*c*) *In Sulphuric Acid* 25 *per cent.* Pour on a few drops of the acid, and, after letting it act for half a minute or so, pour off, and then place the slide with film in a wide-mouthed bottle containing 25 per cent. H_2SO_4 for at least sixteen hours, but not longer than twenty-four hours. Wash thoroughly, counter-stain with weak, watery solution of methylene-blue for a minute or so, dry and mount in oil or balsam. The only bacilli remaining red are the tubercle bacilli.

9. In cases in which, after trying the differentiating methods just described, doubt occurs as to the organism under consideration being the genuine or pseudo-tubercle bacillus, two other methods should be resorted to : first, the cultivation, and, second, the inoculation test.

(1) The secretion to be examined is mixed with nutrient 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 real bacillus of tubercle requires a higher temperature and a longer time for its growth. This method has many practical difficulties.

(2) *Inoculation.*—An animal known to be very susceptible to the tubercle bacillus, such as the guinea-pig, is injected intra-peritoneally with the secretion. After a month to six weeks, if the animal lives as long, it is examined.

This method is conclusive, but it has the disadvantage that the time required before a result can be obtained is long, and that a special license is required. The former objection is a serious one 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 organism found (in milk, for example) is the tubercle bacillus or not. Nothing less will satisfy us" (p. 505).

I think that the differential staining method which I have described, based on the relative time required for the decolourisation of the pseudo and genuine tubercle bacilli, will meet that want, but only time and further confirmation will prove whether this is as constant and reliable in other hands as it seems to be in mine.

My researches have proved conclusively that the usually described decolourising or staining methods for differentiating the pseudo (especially the smegma bacillus) from the genuine tubercle bacillus are absolutely fallacious.

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The Examination of Sputum for Tubercle Bacilli.

The expectoration obtained from cases of phthisis varies very considerably in quantity, nature, and consistency. It may be very abundant or very scanty, liquid or viscid, mucoid, muco-purulent or purulent, and very little reliance is to be placed on the naked eye appearance. Not infrequently I have found tubercle bacilli in the most unlikely looking frothy sputum.

Preferably the expectoration which is brought up the first thing in the morning should be chosen, and in cases in which there is little expectoration it should be particularly pointed out to the patient that it is the phlegm that is brought up by a cough which is required, not that merely hawked up from the throat.

Such sputum should be sent in a clean, wide-mouthed bottle, or some such vessel, and should have no antiseptic added to it. I am not convinced that sputum in carbolic lotion is more difficult to examine, but I prefer it without this addition, as it is much more difficult to pick out the suitable pieces, as the carbolic acid coagulating the albumen turns it white. Sometimes, especially in nervous women, it will be necessary to obtain their expectoration unknown to them from their handkerchief, whilst in young children, who seldom expectorate, but usually swallow their sputum, it may be necessary to examine the contents of the stomach, obtained by lavage during fasting, or even the fæces, as suggested by Macé.

The sputum should be examined as soon as convenient, but tubercle bacilli may be found in

expectoration that has become putrid, but I do not think they stain quite as well as in fresh specimens.

Selection.—A point of the very greatest importance is the selection of the part for microscopic examination, and a little care taken in this, the most essential part of the whole process, may save a great deal of trouble and not infrequently prevent a negative result.

The expectoration, when received, should be poured out into a Petri dish, and this placed on a black background, or as I prefer, into a small black photographic developing dish, or if the specimen is not too liquid, on to a black piece of paper.

The last has the advantage that after use it may be thrown into the fire, and one is saved the trouble of washing the dish. Spread out in this way on a dark background, it is easy to pick out the opaque whitish grey or yellowish nodules, which are especially suitable for examination. These small, somewhat yellow, lenticular particles come from the wall of a cavity, and may contain an enormous number of bacilli. These nodules are, however, often absent, and in these cases the most purulent part of the sputum, avoiding as much as possible the transparent mucus, should be chosen. I have occasionally taken by mistake opaque whitish particles, which have turned out on microscopic examination to be particles of food, milk or starch, which have been entangled in the expectoration in its passage through the mouth, so that it is advisable, in at least doubtful cases of tuberculosis, to ask the patient to send a specimen obtained before food has been taken, or even to rinse out his mouth before expectorating. These may seem unimportant details, but they have not appeared so to me.

Spreading the Films.—The selected piece about the size of the head of a wax match, may be picked out of the sputum by means of two needles fixed in handles, by two clean nibs in penholders, by forceps, or even by two wooden matches. This is then placed in the middle of a clean slide, and another applied to it flat, but at right angles to the first. By rubbing these to and fro, and then rapidly sliding them apart, a uniform and fairly thin film is obtained on the lower slide. The film, according to some writers, should not be too thin; but my opinion is that most film preparations are too thick. It is well to try and obtain a film very thin at one end and slightly thicker at the other.

Sometimes, especially when the sputum is very liquid and contains much saliva, it will not spread out well. This difficulty may be easily overcome by slightly warming the slide before or during the spreading.

Two or three films are prepared in this way, and allowed to dry in the air before being fixed.

Drying may be hastened by holding them over the flame and fixation completed by heating the slide till it is fairly hot. They may whilst still hot be stained.

Staining.—I place the slides on a piece of wood about six to eight inches long, and two inches broad and deep, and, whilst still hot, filter on Ziehl-Neelsen's carbol-fuchsin, which I keep in a bottle fitted with a very small funnel, in which a piece of filter paper is kept. The whole area of the film for about one inch square is covered with the stain, and after a few seconds, holding the slide at the extreme end with the thumb and finger, as level as possible,

I warm it again till steam rises for a few seconds, but take care not to allow actual boiling or drying up of the stain to occur, otherwise a granular deposit of the dye takes place. The films are again placed on the wooden block, and allowed to stain for about seven minutes. The exact time they stain does not matter very much, but should not be less than one or two minutes. By this time the films are cool, and they are then well washed in tap water, and placed in 25% sulphuric acid, which I keep in a wide-mouthed bottle (Fig. 1, page 5). Decolourisation may be hastened by moving the films about in the acid, or by replacing the stopper and shaking the bottle. In a few seconds the preparation is washed in water, when the film, which has become a yellowish brown colour by the acid, turns somewhat red. It is again replaced in the acid for a short time, and the treatment with water and acid repeated until the film is practically colourless, or has only a faint lilac tint, and it is then ready for contrast staining. The duration of the treatment with acid depends on the thickness of the film and the number of tubercle bacilli present, but usually the preparations are colourless after two or three rinses in the acid and then in the water. Occasionally, but very rarely, it happens that the film, after repeated immersions in the acid, still remains a distinct red colour, and one is apt to conclude that it is insufficiently decolourised. This will be found to be due to the fact that the tubercle bacilli are so numerous that even to the naked eye the preparation appears red. The experiments previously described show that one need not be anxious that the tubercle bacilli themselves may be decolourised by prolonged treatment with the acid. I found that in preparations of

sputum stained as described, the tubercle bacilli remained distinctly red after 72 hours' immersion in 25% sulphuric acid. After the films have been decolourised they are well washed in water, and immersed for a second or so in a wide-mouthed bottle containing about a 1% solution of methylene blue. I use any methylene blue solution which may be at hand. Löffler's or Kühne's solutions will do very well, as the only object in view is to stain the whole film a blue colour. They are then washed in water, dried, and examined in immersion oil. The tubercle bacilli and other acid-fast organisms are stained red, the rest of the preparation, including all other organisms present, a blue colour, varying in intensity according to the duration of immersion in, and the strength of the solution of methylene-blue. The whole process takes but a few minutes, and the fingers are not soiled when it has been carried out as described. The formula for Ziehl-Neelsen's carbol-fuchsin has been given before (page 61). Gunther insists on the importance of using distilled instead of tap water for making the solution. The carbol-fuchsin solution is very permanent, although, usually after a month or so, an oily substance separates out, but this does not interfere with its use, provided the solution is always filtered before staining.

FRANKEL-GABBET's MODIFICATION OF ZIEHL-NEELSEN's METHOD.

This, the only other method of staining tubercle bacilli in sputum in general use, differs from Ziehl-Neelsen's process in that the decolourising and after-staining are done at the same time by one solution.

The preparations, after being stained with carbol-fuchsin, as above described, are washed, and then

placed in a solution consisting of methylene-blue one part, and 25% sulphuric acid 99 parts, and are allowed to remain there from two to four minutes. They are then washed, dried, and examined.

I think that, although this method is convenient, it is better to carry out the decolourising and after-staining separately.

PRACTICAL CONCLUSIONS DERIVED FROM THE EXAMINATION OF THE SPUTUM.

It must be remembered that in miliary tuberculosis, sputum is often absent, or has merely the character of that from a case of bronchitis, and tubercle bacilli will be rarely found in it.

In cases of hæmoptysis, the number of bacilli found in the expectorated blood, especially when this is very abundant, is, as would be expected, very small, and the detection of them difficult, often impossible. The bacilli will be most easily found in the little solid pieces of sputum streaked with blood which the patient often brings up a few days after a hæmorrhage, and here they may sometimes be found in large numbers, even when no physical signs can be detected in the lung.

The number of tubercle bacilli found in pulmonary tuberculosis has in general no relation to the gravity of the case (Czaplewski), for there is no relation between the number expectorated and the number of bacilli which may be present in the tissues. (A. Fränkel).

It not infrequently happens that in a phthisical patient in fairly good health, who has recently had an hæmoptysis, the sputum may contain a very large

number of bacilli, without their presence seriously impairing his general health, or causing fever. On the other hand, tubercle bacilli do certainly increase in number in sputum, when there are signs of increased breaking down of lung tissue, and also diminish when a cavity shows physical signs of healing.

Hardly any prognostic indications can be drawn from the size, form, or peculiarities in staining of the bacilli. Whether the bacilli occur in the form of short rods, or in segmented, chain-like forms, or whether they take on the stain evenly or unevenly, matters little. These may alike be seen in the slight and in the serious cases—in those who are recovering, as well as in those who are doing badly.

The small masses of bacilli clubbed together in a a dense felt-work almost always, according to Wurz, come from the wall of a cavity, whilst bacilli with so-called spores, *i.e.*, the beaded form, are most frequently found in sputum which has come from this position. (Cornet and Meyer).

I have more than once noticed that in sputum from cases of tubercular laryngitis, the bacilli often occur in small masses nearly all the elements of which are beaded.

Next to the presence of the tubercle bacilli, the most important indications are to be derived from the associated organisms found in the sputum.

The most frequent, according to Cornet and Meyer,* is the streptococcus pyogenes which often occurs in very long chains, consisting of over 50 cocci. The presence of these gives a bad prognosis. (Spengler). Next in frequency is the staphylococcus

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*Cornet and Meyer: "Tuberculose." Handbuch der path. Mikro-organismen. Vol. II. p. 176.

aureus and albus, and then the micrococcus tetragonus, diplococcus pneumoniæ, the bacillus of diphtheria, and the influenza bacillus.

As Lehmann and Neumann† say, the streptococcus plays an important rôle in phthisis, it accompanies the specific cause and markedly influences the appearance and course of the disease. Hectic fever is strepto-coccus fever. (Petruschky).

In a general way the bacteria which accompany the tubercle bacilli may be recognised in films stained in the ordinary way for the specific organism, but in many cases it is advisable to take some care in the selection of the sputum. The examination of the sputum gives us a clue as to the bacterial flora of the lungs, but as one part may be secondarily affected, another unaffected, and a third part affected with other bacteria, it is essential to examine several portions of the sputum before giving an opinion.

The pus-producing organisms occur in little balls in the sputum, and some of these opaque nodules should be picked out, washed repeatedly in sterile water, at least ten times according to Koch and Kitasato, and films made from the centre of these.

Such films should be stained with the most suitable reagent for showing the structure of the organisms: the strepto- and staphylo-cocci by any of the ordinary dyes, *e.g.*, methylene-blue; the pneumo-cocci and influenza bacilli, with dilute fuchsin, as will be described later.

Having regard to the numerous cases recorded by eminent bacteriologists, as previously mentioned, of the possibility of finding acid-fast bacilli in sputum in cases which were definitely non-tubercular, are we

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† Lehmann and Neumann. Bacteriology, p. 139.

justified in considering bacilli which retain the stain after being subjected to decolourisation by 25% sulphuric acid, to be genuine tubercle bacilli? In answer to this question, I would say, in by far the majority of cases, yes; but not absolutely. What then are the indications which would suggest that we are dealing with a pseudo-, and not a genuine tubercle bacillus in sputum? If the bacilli in question do not conform to the general description already given of Koch's bacilli; if they are very short, or very thick, or occur in long branching filaments, instead of appearing as delicate, graceful rods; if the organisms stain a bluish red, instead of a brilliant red; if, in other words, they seem in any way atypical, and particularly if the clinical signs and progress of the case do not warrant an assumption of tubercle, then it is highly desirable to at least resort to the differential methods I have previously described in detail.

How frequently or how seldom the pseudo- have been mistaken for the real tubercle bacillus, no one can say; but I cannot but think that the examination for the tubercle bacillus should not be merely a colour analysis, attention should also be drawn to the general aspect and morphology of the organism. In sputum, I think, an error in diagnosis will be seldom made; on the other hand, in the examination of urine, fæces, etc., the possibility of confusion is very real, and certainly not infrequent, and the method of differential diagnosis of the greatest possible importance.

EXAMINATION OF SPUTUM BY SEDIMENTATION METHODS.

In cases in which no tubercle bacilli can be found in the sputum of a suspected case by the ordinary

method of examination, various procedures have been suggested for rendering the sputum liquid, and after subjecting this to sedimentation or centrifugalisation, to examine the sediment for the bacilli.

Biedert's Method.—Two table-spoonfuls of water and 15 drops of liquor sodæ are added to one table-spoonful of sputum, and the resulting mixture is thoroughly stirred and then heated. A further four table-spoonfuls of water are added, and it is again heated until the mixture becomes liquid and homogeneous, in which only a few particles are seen floating about. Three to six table-spoonfuls of water are added, and the mixture, placed in a conical glass, is left to settle for 24 to 48 hours. At the end of this time film preparations are made from the sediment. If the latter cannot be spread, or does not stick to the glass, a little of it may be mixed with some of the original sputum or with glycerine albumen, and films are then made in the ordinary way.

Ketel's Method.—This is much easier than Biedert's. A mixture consisting of 100 cc. water, 6 cc. of carbolic acid, and 10 to 15 cc. of sputum, is thoroughly shaken for one minute. The resulting solution is poured into a conical glass and allowed to settle for 12 to 24 hours, when films are made from the sediment as before.

In both methods it will be found advisable to fix the films by immersion in absolute alcohol for a few minutes, to ensure their adhering to the glass slide.

I have had little experience with any of the sedimentation methods of examining sputum, as I prefer to select suitable portions and examine several films in cases in which they are not at first found.

The finding of the true tubercle bacillus settles the diagnosis, but a negative result does not exclude tubercle. I have in one suspicious case examined the sputum thirteen different times before I detected a single bacillus, so that in cases in which the disease is suspected it will be necessary to make repeated examinations on different occasions before being satisfied that Koch's bacillus is absent.

The Examination of the Urine for the Tubercle Bacillus.

This will be necessary for the recognition of tubercular disease of the bladder and kidney. " The urine of pronounced renal tubercle is characteristic. It is light in colour, murky with mucus, depositing a fine layer of pus and a few caseous clumps. It is always albuminous. It is faintly acid or neutral, and of medium specific gravity. Bacilli are discoverable, and if the urine is injected into guinea pigs subcutaneously, a typical tuberculosis is produced in a fortnight or three weeks. Later, as the disease advances, putrefactive bacteria cause an offensive odour, and muco-pus in large quantities is passed ; pus increases in proportion to the grade of pyelitis. (Hurry Fenwick).*

The determination of the origin of the tubercle bacilli, whether from the bladder or kidney, or from both, must rest on the nature of the other elements found in the urine, and on the clinical signs and symptoms of the disease.

I have not found the detection of the tubercle bacilli in the urine as difficult as might be imagined, and am

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* Hurry Fenwick : Encyclopædia Medica. Vol. vi., p. 71.

convinced that the detection of them in obscure diseases of the urinary tract will prove of the very greatest help in diagnosis, and will, in some cases, at least, lead to the cure of the disease.

I must here draw attention to the importance of not mistaking the smegma bacillus for the genuine tubercle bacillus. This accident has happened at least three times to my own knowledge.

One was a boy, in whose urine so-called tubercle bacilli were found repeatedly. Films made from the urinary sediment were sent to two leading English bacteriologists, and they confirmed the diagnosis of tubercle. In fairness to these, I should say that, as far as I know, an actual specimen of urine was not sent for inoculation experiments. The parents of the child were informed of the serious nature of the illness, and the child was sent abroad. Whilst there, either he came under the care of, or the urine was sent to, Coppen-Jones, whose work on tubercle is well known. He found that the so-called tubercle were only smegma bacilli, and the after history of the case proved this correct.

The two following cases occurred in my own practice: A little girl had a sudden attack of hæmaturia, with a large amount of albumen and tube casts in the urine—evidently a case of acute nephritis. In film preparations of the urinary sediment, I found numerous acid-fast bacilli, which, in so far as they were not decolourised by alcohol, which, as nearly all the text-books still say, distinguishes the smegma from the true tubercle bacillus, I thought there was an underlying tubercular condition. Unfortunately, this case occurred before I had worked out the resistance of the acid-fast bacteria, and, therefore, I

was not able to subject films to the tests which I have since found answered so well in other cases.

A fresh specimen was sent to Prof. Woodhead, who kindly examined it by inoculation experiments, and, after six weeks, reported that there were no signs of tuberculosis in the injected guinea-pigs.

Another case, a servant aged 35, with a very strong family history of phthisis, had chronic cystitis. In her urine I detected what I thought were tubercle bacilli. She was sent to her home, Glasgow, with a view of having an expert opinion. The surgeon, an authority on genito-urinary diseases, confirmed my diagnosis of tubercle, and his opinion was corroborated by a leading bacteriologist, who also detected tubercle bacilli. We all gave a very bad prognosis then—*i.e.*, four years ago. She came back to Bournemouth again under my care, and has gradually gained in weight and has lost her symptoms, and, in fact, she is now in better health than she has been for years. After my research work on the acid-fast bacteria, and after I had discovered that all the methods for differentiating the smegma from the tubercle bacillus usually described, were, at least in my hands, absolutely unreliable, I have frequently examined her urine and still find numerous acid-fast bacilli, but these are decolourised by immersion in 25% sulphuric acid before 24 hours, or in the rosolic acid mixture before six hours.

I admit that in these two cases, the urine has not even been put to the inoculation test, and as neither has died, crucial evidence of their non-tubercular nature may still be considered wanting.

On the other hand, I have within the last six months found the genuine tubercle bacilli in the urine

of three cases, and these bacilli withstood the long decolourisation with 25% sulphuric acid and the rosolic mixture. One of these has recently had her left kidney removed by Hurry Fenwick, who stated that it was a typical tubercular kidney. In the other two cases the urine was sent to me for examination without any clinical history. After I had definitely reported on the presence of Koch's bacilli, I learnt that one had tubercular caries of the spine; the other extensive tubercular disease of the lungs.

Cornet and Meyer[*] state that the most usual situation for the smegma bacillus is in the preputial sac and the cleft between the large and small labia; they are, however, also found in the region of the external genitals and anus generally, and in the folds of the groin, in cerumen (Gottstein and Bitter), on various parts of the skin (Laabs), at all the natural openings of the body, as well as on the tonsils and in the coating of the teeth and tongue.

" Such being the favourite position of the smegma bacillus, it is only natural that it should be found especially in the urine and fæces, and its presence here has occasioned operative interference, and even fatal mistakes. This is shown in a case of Laabs, in which, on account of the presence of organisms which were considered to be tubercle bacilli, in the urine, an operation was undertaken, and instead of the supposed tubercular kidney, an abscess in the lumbar region was found.

" Mendelsohn, Konig, Bunge and Trantenroth have described extirpation of the kidney in cases in which the operation was performed from a mistaken diagnosis from the same cause.

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[*] Cornet and Meyer: *loc. cit.*, p. 93.

" Drawing off the urine by means of a catheter will certainly afford entire protection against the presence of the smegma bacillus."

I cannot agree with the last statement, which is also very generally made in the text-books. In the male, if the glans is washed before micturition, the urine will, in my opinion, be found quite as free from the smegma bacillus as would be the case if a catheter had been passed ; whilst in the female, the amount of smegma lying in this region is often surprising, and a catheter passed in the ordinary way, by the sense of feeling and not by sight, is much more likely to take into the bladder a larger quantity of smegma than would be found had the urine been voided in the ordinary way. If the parts had been thoroughly cleansed before the act, then my impression is that the catheter is not necessary. At all events, I do not think that, now we have a means of distinguishing the pseudo- from the genuine tubercle bacillus, any special precautions are necessary.

The following is the method I always adopt :—About eight to ten ounces of urine, passed in the morning, is collected in a clean bottle. It is well to examine the specimen as soon as convenient after it has been passed. This is well shaken up and poured into a conical glass. For this purpose I use a conical specimen glass, made by Baird and Tatlock, which only differs from that in ordinary use in
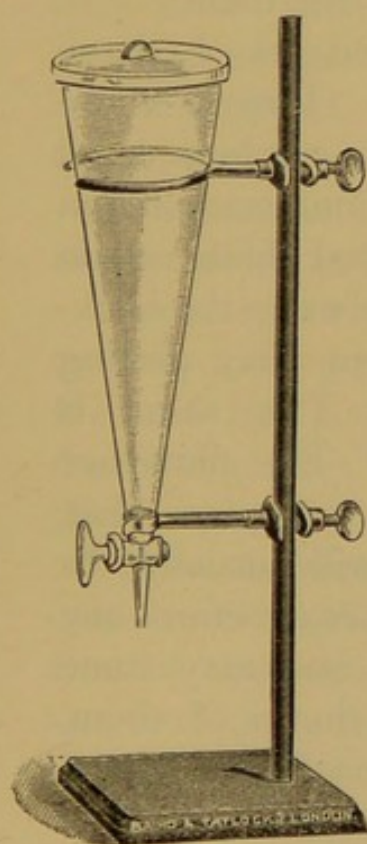
FIG. 3.

having a glass tap at the lower end and a loose cover on the top. The one I use holds about 280 cc. It is placed in one of the rings of a retort stand, and is much more convenient than having to pipette off the sediment, and it can be more easily cleaned.

The urine is allowed to settle for twelve to twenty-four hours. At the end of this time, by gently turning the tap, a drop or two of the sediment is allowed to run out, and is received on the centre of some half-a-dozen slides. This is then spread out by a needle, and the slides are allowed to dry in the air. I do not think it advisable to hasten the drying by heat, as the albumen in the urine may be coagulated, and the specimens become opaque and cracked. When the films are quite dry, it is well to place them in alcohol, or alcohol and ether, for a few minutes to thoroughly fix them.

All the six films are now stained as I have described, and then washed in water. Two of these I subject to decolourisation with 25% sulphuric acid for a few minutes, just as in the routine examination for sputum. I may here remark that films made from urinary sediment do not decolourise well; the background remains somewhat reddish, even after placing in the acid for one or two hours. The same is true often of films of smegma itself. The films are then washed, counter-stained with methylene-blue, dried, and examined in oil. It will usually be necessary to go over rather a large area before any red-stained bacilli are seen, and then one may come across an isolated bacillus, or often a clump of them. It is said that in urine the tubercle bacilli are in S shaped aggregations (von Jaksch), or in tufts.

When such bacilli are delicate, not plump and thick,

and often beaded, I think they will prove to be the genuine Koch's bacilli.

Having found bacilli stained red, the next thing is to determine whether they are the smegma or tubercle bacillus. For this purpose, after taking off the excess of the stain in two of them by pouring on a little 25% sulphuric acid, I drop these into that acid kept in a wide-mouthed bottle, and leave them undisturbed for at least sixteen, and not longer than twenty-four, hours. I treat the remaining two slides in the same way, only with the rosolic acid mixture, with or without the methylene-blue, and then place them in that mixture for at least four, and not longer than twelve, hours In each case they are taken out at the end of that time, well washed in water, and counter-stained in watery methylene-blue, except when they have been placed in the rosolic acid containing methylene-blue, in which case they are already counter-stained. The films are now washed in water, dried, and examined in oil.

Any bacillus which remains stained red after this, I am satisfied, is the genuine bacillus of tubercle.

Meyer has suggested the addition of a little thymol to the urine, in order to retard fermentative changes. Kirstein has proposed that the urine should be passed through a filter, and from the deposit remaining on it films are made.

Many bacteriologists prefer to centrifugalise the urine. If this contains much pus, it may be first treated by Biedert's method, as described under sputum, and then centrifugalised, or subjected to sedimentation.

If urates are very abundant, they can be dissolved by heating the preparation before centrifugalising, or

by the addition of boric acid and borax, or simply by the addition of a few drops of acetic acid.

Strasburger adds two or three volumes of alcohol to the urine, in order to hasten the process of sedimentation.

Trevithick* believes that the urinary salts in the urinary deposit prevent the pus cells and bacilli from adhering sufficiently well to the slide to withstand the subsequent treatment of staining, etc., and thinks that the majority of the bacilli get washed away. He, therefore, advises that the sediment from the conical glass be centrifugalised, after which the supernatant fluid is decanted, and distilled water added to and shaken up with such deposit. This process of washing with distilled water, and subsequent centrifugalising, may be repeated once or twice, and films prepared from the final centrifugalate.

I have seen it stated, but cannot remember where, that if in very clear urines a few tiny shreds of cotton wool are added, these will, whilst settling to the bottom, often form nuclei to which the bacilli become attached, and the examination of these will facilitate the detection of the bacilli. Fenwick states that there is an impression, neither proved nor accepted, that tubercle bacilli are more easily and more abundantly found in renal than in vesical tuberculosis. He says: " When the urine is decomposing in the bladder from septic cystitis, tubercle bacilli are not found," and that after a course of bladder irrigation, or soon after a course of injection with Koch's new tuberculin, they are not found, or are only discovered with difficulty.

Fenwick further remarks that the presence of tubercle bacilli in clear, sterile urine is not

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* Trevithick: *Brit. Med. Journal*, 1904. Vol. i., Jan. 2, p. 13.

pathognomonic of urinary tuberculosis, for they have been found in the urine of cases of phthisis and tubercular bone or joint disease. If, however, they are found in the pyuric urine of patients who have urinary symptoms, they, no doubt, are answerable for tubercular disease in the urinary tract.

Maragliano[*] has examined the urine of twelve cases of pulmonary tubercle, in which the urinary tract was unaffected, to see if tubercle bacilli could be detected. Although several methods were tried, the result was completely negative in every case.

Injections of the centrifugalised fluid proved sterile in guinea pigs.

In cases of doubt, recourse should be had to inoculations, which are usually successful, in early tubercular urine; but later, when the cystitis has become purulent, and pyogenic organisms have been added to the tubercle bacilli, the results are not so good. (Wurtz).

The Examination of the Fæces for Tubercle Bacilli.

Tubercular ulceration of the intestine occurs most frequently in the cœcum and lower part of the ileum, and is said to diminish in frequency above and below this point. Primary tubercular disease of the intestine is commonest in children but also occurs in the adult, whilst secondary ulceration is not infrequently associated with pulmonary disease.

Intractable diarrhœa occurring in the course of phthisis is probably due to waxy disease or to ulceration, or to both, and in association with the other important

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[*] *Brit. Med. Journal*, 1904. April 9. Epitome.

clinical signs, the demonstration of the tubercle bacillus may give considerable help.

In addition to the difficulty in many cases of finding the bacilli in the fæces, it must be remembered that tubercle bacilli found in this situation in phthisical patients may be merely derived from the sputum which has been swallowed. Examination of the fæces for tubercle is even recommended as a method of detecting pulmonary tubercular disease in young children, who do not expectorate but swallow the sputum.

Bodo* examined post mortem the intestinal contents of nine cases of phthisis, and although no signs of tubercular disease of the intestine were detected, yet he found the tubercle bacilli in three of these. This was probably due to the fact, as Cornet and Meyer point out, that just before or during the time that death took place, the expectoration had been swallowed.

Tubercle bacilli found in fæces are only of diagnostic importance (i.) when we can be sure that the patient, if he has tubercular disease of the lungs, has not swallowed his sputum, (ii.) when we repeatedly find the bacilli present, and (iii.) when the clinical symptoms support a suspicion of tubercular disease of the gut.

Another important point to be remembered is, that acid-fast bacilli closely resembling the tubercle bacilli in general size, shape and staining reaction, are often, if not usually, to be found in the fæces in health, and films must therefore be decolourised by the method which I have described, before we can definitely say that we have found the true tubercle bacillus.

In order to examine the fæces it is advisable to look for any mucus or purulent masses, and spread several

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*Cornet & Meyer: "Tuberkulose:" Handbuch der path. Mikro-organismen. Vol. ii., p. 88.

films from these. If these are absent we may give the patient some preparation of opium as suggested by Rosenblatt, in order to get a formed stool, and then to pick from that any mucous shreds which may be adherent to it. This is more particularly successful when the ulceration is situated in the lower part of the large intestine. Strasburger, after removing the larger fæcal masses, shakes up the rest with one or two volumes of 96% alcohol and then centrifugalises this.

The following case in which I easily found the tubercle bacilli in the fæces quite recently will show the method I adopted. Professional man, aged 35, with cavity in the lung, but no signs of active disease present. Tubercle bacilli fairly numerous in the expectoration. For the last six months he has had diarrhœa. There is no enlargement of liver, or spleen, and no albumen in the urine.

The fæces received for examination were of the consistence of pea soup. As no suspicious particles were seen, films were made from a little of the material taken at random, dried in the air, fixed in alcohol for a minute or so, and stained and decolourised in the ordinary way. Brilliantly red stained bacilli were seen in small numbers in two films, sometimes isolated, sometimes in small groups. They were delicate looking rods and showed the beaded appearance.

In order to be sure that they were not pseudo-tubercle bacilli, one stained film was placed in 25% sulphuric acid for 22 hours, another in the rosolic acid solution for six hours. In both these preparations red stained bacilli were distinctly and easily seen, and there is no doubt that they were the genuine tubercle bacilli.

As the patient informs me that he never swallows his expectoration, which is small in amount, as they were found in each subsequent examination, and there is marked diarrhœa and no signs of waxy disease, I think one may be justified in diagnosing tubercular ulceration of the intestine, secondary to pulmonary disease. I may here mention that within fourteen days from the time when I detected the tubercle bacilli in the fæces, the patient rapidly developed abdominal symptoms, and died from collapse.

The Examination of Pus.—Pus from a chronic tubercular abscess is generally a whitish sero-purulent liquid, which soon separates, on standing, into a powdery sediment with a serous layer above. Tubercle bacilli may sometimes be recognised in it, but I have more frequently failed to find them in undoubted tubercular abscesses.

In all tubercular *serous* effusions, *e.g.*, a pleural fluid, it may be stated that as a rule the detection of the bacilli, even after careful centrifugalisation or sedimentation, will be very difficult and often impossible. In sero-fibrinous pleurisies the examination for Koch's bacillus is often negative. It has only been found three times out of 20 cases by Fernet, twice out of nine cases by Ehrlich, and Peron has only met with it once. (Labbé). Some diagnostic information may be gathered by a cyto-diagnostic examination of the sediment (see later).

In lupus, although this is undoubtedly a tubercular affection, tubercle bacilli are very scanty and will not be often found.

The Gonococcus.

MICROCOCCUS OR DIPLOCOCCUS GONORRHŒÆ.

The organism is to be most frequently looked for in the pus from gonorrhœal inflammation of the urethra of the male, the urethra, cervical canal and vulva of the female, and in gonorrhœal ophthalmia.

In order to obtain specimens for microscopic examination one should proceed as follows—

In the Male when there is a fairly profuse discharge, the pus at the anterior end of the meatus is cleared away, and by pressure on the urethra fresh pus is forced forward from the deeper parts, by this means a drop is obtained with as little contamination as possible. This is allowed to impinge on one end of a clear slide, and with the aid of a second slide is spread out in a thin uniform layer. The preparation is then allowed to dry in the air and can be stained at once or at any time subsequently. When, however, the discharge is very slight in amount, it is wise to use the first drop of pus that appears at the orifice of the urethra, as it is sometimes impossible to procure any more even by pressure along the urethra.

When the disease has become chronic, *i.e.*, a gleet, it is sometimes by no means easy to obtain any discharge at the time when the patient presents himself. In these cases or in the convalescent stage of the acute disease, it is a good plan to give the patient two glass slides, explaining to him how to make smear preparations, in order that he may make a film at any such time as he may find any discharge.

It may be necessary in some cases to examine the urine for gonococci. Here the organisms are chiefly

found in the prostatic threads, and by allowing the urine to stand in a conical glass or by centrifugalising it, film preparations can be made from the sediment in which the gonococci associated with other organisms may be found.

It is well to remember that although no gonococci may be detected in the thin discharge from the urèthra in cases of gleet or apparently cured gonorrhœa, yet it is not safe to assert that the patient is free from infection. In such cases an acute attack may occur without fresh infection, and gonococci may then be found in large numbers in the pus. It has been suggested that an injection of silver nitrate may be used to excite such an inflammation, in order that the resulting discharge may be examined microscopically.

" Swabbing out of the urethra with silver nitrate so as to set up an acute urethritis, as practised by American surgeons especially, will often bring into evidence gonococci concealed in the follicles of the urethra, long after the original discharge has ceased." (Curtis.*)

Foulerton,† in his excellent account of the gonococcus, says : — " Gonococci may sometimes be found in the so-called ' prostatic threads,' which are passed at the end of micturition, long after they have disappeared from any gleety discharge which may exist. In some cases also the presence of the cocci may be ascertained by practising ' expression of the prostate.' This proceeding is carried out as follows : The patient almost, but not quite, empties his bladder, the prostate is manipulated by means of

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* Curtis : Practical Bacteriology. 1900, p. 124.

† Alex. Foulerton : "On micrococcus gonorrhœæ and gonorrhœal infection." *Trans. of the Brit. Institute of Preventive Medicine.* First series, 1897, page 40.

a finger introduced into the rectum, and, finally, the remainder of the urine is expelled. This residue will contain a considerable quantity of the prostatic secretion, in the cells of which cocci may be detected."

In the Female, gonococci are to be looked for most frequently in the urethral or vulvar discharge, less commonly in the discharge from the cervix uteri, and rarely or never, according to most observers, in the vaginal discharge.

Foulerton states that the discharge from the cervix is the most common source of infection for men, for, whilst urethritis in women is not usually of long duration, gonorrhœal inflammation of the cervix is very persistent, and often exists without any obvious symptoms,

Herman,[*] however, says that the gonococci linger longest in the urethra, probably in the glands in the floor of the urethra near the meatus, and next longest in the cervical canal.

In order to obtain pus for examination from the female, the best plan is to examine the patient some time after micturition, and squeeze the discharge out of the urethra by pressing with the finger in the vagina upon the urethra from behind forwards. If none be obtained in this way, it is advisable to put in a small, sterilised, blunt curette or director, and draw it along the lower wall of the urethra from behind forward.

A specimen of the discharge can be obtained from the cervix by using a piece of wool fixed on a probe or sound, or, as Foulerton suggests, by means of a capillary tube, passed through the speculum with

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* Herman : Diseases of Women. 1898, p. 435.

forceps. If such a specimen is contaminated with the vaseline on the speculum, this is removed by placing the air-dried films in ether for some minutes.

Film preparations can also be made from the discharge in cases of gonorrhœal conjunctivitis, from the pus in cases of pyosalpinx, and sometimes gonococci may be found in cases of inflammation of Bartholin's gland.

THE APPEARANCE OF THE GONOCOCCI.

The micrococcus gonorrhœæ, or gonococcus of Neisser, usually occurs in the form of diplococci—*i.e.*, two somewhat oval cocci lying in contact with one another. (Plate I. Fig. 2.) The surfaces opposed to each other are either flat or slightly concave, whilst the outer surfaces are convex, so that there is a clear unstained space between the cocci, generally slightly oval in form. Each coccus is, therefore, somewhat kidney-shaped, and the appearance of the diplococci is very aptly compared to that of two coffee beans lying side by side.

When multiplication by fission takes place a tetrad is formed, but the latter is not nearly as frequently seen in pus as the diplococci form, although often met with in cultures.

The gonococci vary somewhat in size. Bumm gives the following measurements :—Large diplococci—length from pole to pole, 1.6μ ; breadth in the centre, 0.8μ. Very small diplococci, in which the division into two is only slightly marked—length, 0.8μ ; breadth, 0.6μ. He says that the majority of associated pairs measure over 1μ in breadth.

Foulerton gives the measurement of the individual cocci as 0.7μ long, and about 0.5μ broad. He also

states that sometimes one of a pair of cocci is distinctly larger, and stains more deeply than the other. Macé* notices that under a high magnification the cocci appear distinctly asymmetrical.

For diagnostic purposes the actual measurement of the gonococci is of very little use, as the size of the organism depends to some extent on the method of fixing and staining employed.

Very young diplococci are smaller than the fully-grown coffee-bean gonococci, and often assume the figure of 8: whilst in faintly-stained preparations the organisms appear smaller than when deeply stained.

Situation: The gonococci are found in three positions: First, and most characteristically, in the protoplasm of the leucocytes; second, in the epithelial cells; and thirdly, free in the mucus secretion.

Neisser was at first of the opinion that the gonococci only adhered to the pus cells, but Haab pointed out that they were inside the protoplasm, and this has been generally confirmed. Sometimes the leucocytes are seen crammed full of organisms, and often the pus cells, which are larger and more swollen than the normal, appear to be on the point of, or actually bursting. It is not unusual in stained preparations to see that the pus cell has ruptured, and scattered its contents of gonococci, probably owing to the pressure used in spreading the film. It is stated that Bumm counted as many as two to three hundred diplococci in a pus cell distended to the point of bursting, whilst a hundred may often be seen.

Foulerton remarks that the number of cocci in a pus cell will nearly always be found to be in the series of 4, 8, 16, etc.

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* Macé: Traité de Bactériologie. 1901, p. 388.

Seldom, if ever, do the gonococci actually penetrate the nucleus, although they may indent it.

Sometimes the gonococci within the leucocytes may be seen surrounded by an unstained halo or capsule.

The proportion of organisms lying intracellularly and those free, varies not only in different cases of gonorrhœa, but also in different stages of the disease.

Neisser and Scholtz* point out that in the first stage, in which the discharge is mucoid, not purulent, few gonococci are found in the pus corpuscles; on the other hand, many of the epithelial cells are covered with them.

With an increase in the amount of pus in the secretion is associated an increase in the number of intracellular gonococci, so that, when the discharge is quite purulent, the largest number of diplococci are found in the pus corpuscles. As the purulent discharge ceases, there is an increase in the number of extra-cellular gonococci, which lie in the muco-purulent secretion of chronic gonorrhœa, and in the mucus filaments found in the urine.

The free extra-cellular organisms usually occur in little heaps, single diplococci being seldom seen.

Macé,† quoting from the work of Legrain, describes the stages of the disease as follows :—

(1) At the commencement many epithelial cells are seen amongst the pus corpuscles; only 2 to 3% of the latter contain gonococci.

(2) From the end of the second day the proportion of pus cells containing micrococci increase a little; some contain a large

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* Neisser and Scholtz : 'Gonorrhœ.' Handbuch der pathogenen Mikro-organismen. Vol. iii. 1903, p. 154.

† Macé : loc. cit., p. 390.

number — ten to eighty ordinarily; even, according to Bouchard, 120.

(3) The acute period becomes more marked, and the epithelial cells completely disappear. The number of pus corpuscles attacked are greatly increased, one to five or six. At this period the parasite no longer proliferates in the epithelium, but in the deeper layers of the mucous membrane. This is why it is so difficult to reach them.

(4) In the sub-acute stage the epithelial elements become more numerous, but rarely present gonococci; on the other hand, the pus corpuscles which are attacked are present in large numbers.

(5) Lastly, when the gonorrhœa passes into a chronic state the proportion of pus cells is greatly reduced, and it is often difficult to find pus corpuscles among the epithelial elements, but almost all the latter are attacked by the bacteria. Some contain a considerable number—one hundred to one hundred and twenty. The fluid part of the discharge contains a large number of them. Very few are found in the pus cells.

In a case of relapse at the end of five months, the pus reappeared in large quantities, but none of these contained gonococci, whilst the epithelial cells were, on the contrary, filled with them. The inflammation has left the deeper, and returned to the superficial parts, but it no longer presents the same characters as in the acute stage.

It seems to be a disputed point as to whether the pus cells, acting as phagocytes, take up the

gonococci, or whether the latter attack in an active way the leucocytes.

Clinically, there is no practical distinction between the course of a gonorrhœa associated with a preponderance of intracellular organisms, and that of free extracellular gonococci. The statement of Podres and Drobny that a gonorrhœa with extracellular organisms usually takes an unfavourable course has not been confirmed by other observers.

Audry* is of the opinion that complications occurring in the course of gonorrhœa, especially arthritis and synovitis, are due to the presence of organisms other than the gonococci. He says :—

(1) "Every time that I have examined the urethral pus of an individual attacked with blennorrhagia, due to gonococci, unaccompanied by any complication whatever, I have seen associated with the gonococci other micro-organisms, which differed from these in their aspect and distribution.

(2) "I cannot be as categorical on the opposite statement. Yet, in the majority of cases, whe rein the pus from the urethra other micro-organisms are found amongst the gonococci, in all probability we have to deal with a patient who has, who has had, or will have complications. I do not hesitate to consider this a fact of great prognostic value. On the contrary, when the pus only presents gonococci, we may, in the absence of extra-urethral complications, count on its pursuing a normal course."

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* Audry : "Bactériologie clinique du chancre simple et des blennorrhagies compliquées." *Gazette Hebdomadaire*, March 4, 1893.

Preparation of Films.—A drop of pus, obtained as previously described, is placed on a clean slide, and is spread out by means of the edge of another slide, held at an angle of about 45° with the first. In some cases, in which the pus is very thick, it will be found more convenient to gently rub the pus between two slides laid flat. In either case, a thin and uniform film should be obtained. The more pressure that is used the more frequently will ruptured pus cells and extra-cellular gonococci be seen, whereas if the film be too thick the pus cells are not flattened out, and the staining reaction with Gram's method is imperfect.

To obtain the gonorrhœal threads from a specimen of urine, I find the most convenient plan is to pour the urine into the conical glass, described when speaking of the detection of tubercle bacilli in urine, and allow it to stand for some hours. In the absence of this apparatus, it is usually sufficient to place the urine in the ordinary conical urine glass, and after standing for some time, to pipette up the sediment.

Films made from the urinary sediment, or from the prostatic threads, are to be thoroughly dried in the air before fixing.

Such air-dried films may be fixed by passing them two or three times through the flame of a spirit lamp; but a much better plan is to immerse them in absolute alcohol, or equal parts of alcohol and ether, for a few minutes.

If the films are to be stained by Leishman or Jenner's stain, previous fixation is not only unnecessary, but is prejudicial to subsequent staining.

Staining.—The gonococci can be easily stained by practically any of the aniline dyes, whether in alcoholic

or aqueous solution ; but certain stains give a clearer and more distinctive picture than others.

Staining for one or two minutes in Löffler's methylene - blue answers very well, the gonococci being deeply coloured, whilst the protoplasm of the pus cells is almost colourless.

Ziehl-Neelsen's carbol-fuchsin, diluted with water 1 in 10, or carbol-thionine, also gives good results.

Double Staining.—In order to see the relation of the organisms to the protoplasm and nuclei of the pus cells, and to obtain really beautiful microscopic pictures, one of the various methods of double staining may be adopted. The following is one that I often use :—

(1) Fixed films are stained in a solution of eosine—viz., eosine 1 part, alcohol 100 parts, and distilled water 100 parts, for about half a minute ; then washed in tap or distilled water.

(2) They are then stained for a few seconds to one-half minute in methylene-blue solution (Löffler's or Kühne's solution), and are then rapidly washed in *distilled*, not tap, water, dried, and mounted in balsam, or examined simply in oil. The depth of colour can be controlled under the microscope whilst the preparations are wet, with a low power, $^1/_3$—$^1/_6$ inch. Under such magnification the nuclei of the leucocytes should be blue, the protoplasm pinkish, and when examined under the $^1/_{12}$ inch objective, the gonococci stand out as deeply-stained blue bodies in the protoplasm of the pus cells.

Double staining and fixing may be very easily and beautifully obtained by Jenner's or Leishman's stain.

Jenner's stain is made by dissolving 0·5 grams of Jenner's eosine-methylene-blue powder in 100 cc. of pure methyl alcohol (E. Merck's for analytical purposes), and then the solution is filtered.

Leishman's stain can be made by dissolving 0.15 grams of Leishman's eosine-methylene-blue powder in 100 cc. of the same methyl alcohol.

Both stains, or the materials for making them, can be obtained from Baker, 244, High Holborn, or from R. Kanthack, 18, Berners Street. I would also mention that Burroughs, Wellcome & Co. prepare these in "soloid" forms, and these, when dissolved in pure methyl alcohol, are entirely satisfactory.

To use Jenner's stain : A few drops are poured on the air-dried but unfixed film, and allowed to remain two or three minutes, after which the preparation is well washed in *distilled* water.

Leishman's stain is used as follows : A few drops of the stain are poured on the unfixed film, and, by tilting about the slide, allowed to spread over the whole film. At the end of about one-half minute double the number of drops of distilled water are added, and mixed by tilting the slide. The preparation is stained about five minutes, when the stain is well flooded off with distilled water, a few drops of which are allowed to remain on the film for about one-half minute ; the preparation is then dried and mounted in Canada balsam, or examined in oil. In such preparations the nuclei of the leucocytes are stained a ruby red, the protoplasm with its granules a faint red, and the gonococci blue.

GRAM'S METHOD OF STAINING.

One of the most important facts in the differential diagnosis is that the gonococci, unlike most of the cocci, do not stain by Gram's method.

" It has been stated that this characteristic does not differentiate it, as other diplococci have been found in gonorrhœal pus, which react in the same way (Bumm) ; but it is held on equally good authority that the characteristic action of Gram's method on the gonococci is the best and most reliable method of establishing its identity, and that when confounded with other diplococci, the error is due to faulty technique. In our judgment there is no better means than Gram's test for recognising the gonococcus ; but the greatest pains must be taken in every detail to make this test conclusive." (Keyes and Chetwood.)*

Neisser and Scholtz, however, point out that, although the gonococcus in the pus of an acute case, and in pure cultures, is distinctly left unstained by Gram's method, yet in muco-purulent threads, especially from the urinary sediment in chronic gonorrhœa, the reaction is not nearly as constant or as distinct.

The following is Gram's method, as best adapted for diagnostic purposes :—

(1) Stain film preparations, fixed by heat, or better by alcohol, for one to two minutes in aniline-water gentian violet. As this solution will only keep for a short time, I prefer to use carbol-gentian violet. (See page 9.)

(2) After staining, do not wash with water, but remove the surplus stain by means of filter paper, and

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* Keyes and Chetwood : Venereal Diseases.

(3) Treat with Gram's solution of iodine—*i.e.*, iodine 1 part, iodide of potassium 2 parts, and water 300 parts—for about one to two minutes.

(4) Without washing, place the preparation in absolute alcohol until the colour disappears, or until the drippings on filter paper are colourless. This generally occurs in one to two minutes.

(5) Wash with water, and counter-stain with vesuvin, safranin, or weak fuchsin.

Bismark brown may be used as a concentrated watery solution, or better as follows :— Bismark brown 3 parts, alcohol (96%) 30 parts, and distilled water 70 parts. Filter before use.

(6) Wash rapidly in water, dry and mount in balsam or in oil.

The gonococci take on the counter-stain, and appear red when fuchsin has been used, or brown with vesuvin, whilst other organisms, when present, stain with the gentian violet if they retain Gram's stain.

It should be noticed that in the above description the use of water is avoided after stages 1 and 3, and that the alcohol is absolute. Weinrich was the first to point out that the most common source of error in Gram's test was due to the use of water for washing in any of the stages of the process. He found that water interfered with complete decolourisation of the gonococci when subsequently treated with alcohol.

Neisser and Schultz point out that the time necessary for decolourisation in the absolute alcohol to take place depends, amongst other things, on the nature of the film preparation. Gonococci in pure

cultures are decolourised, as a rule, in 15 to 20 seconds; in pus in 20 to 30 seconds; and in preparations made from gonorrhœal threads in about one minute.

The value of Gram's method of staining is shown by the careful experiments of Steinschneider and Galewski. These observers found that in the secretion of the human urethra, diplococci which were decolourised by Gram's stain were only present in 4% of cases, and these could be distinguished from the gonococci by their form and position.

In the secretion of other mucous cavities—*e.g.*, the mouth, nose, rectum, and vagina—diplococci which do not stain by Gram's method may be, however, somewhat more frequently met with.

The meningo-coccus intracellularis (Weichselbaum), which may occur in the nasal secretion, as well as in the cerebro-spinal fluid in epidemic cerebro-spinal meningitis, most closely resembles the gonococcus in its form, position, and staining reaction. It, like the gonococcus, lies almost exclusively inside the leucocytes, and is also decolourised by Gram's method. This organism will be described later.

I will briefly describe two special methods of staining the gonococcus which have somewhat recently been published.

WAHL'S METHOD OF STAINING THE GONOCOCCI.*

This is described as a simple and rapid method, very suitable for clinical diagnosis. The staining solution, which is as follows, is ready for immediate use :—

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* Wahl: *Medical Record, New York*, May 23, 1903, p. 844.

Concentrated alcoholic sol. of Auramin ... 2 cc.
Alcohol, 95% ... ... 1.5 cc.
Concentrated alcoholic sol. of Thionin ... 2 cc.
Concentrated watery sol. of Methyl-green... 3 cc.
Water ... ... 6 cc.

Stain for ten to fifteen seconds.

" The gonococci are stained reddish violet to black ; the nuclei of the leucocytes pale bluish green ; the plasma either colourless or pale yellow, or yellow green, according to the thickness of the film.

" Mast cells are occasionally stained a lilac colour, which, however, is pale, and cannot cause confusion with the gonococci. Urethral bacteria other than the gonococci are either very pale or not stained at all— a point of great assistance in the differential diagnosis. A point of practical importance is that thin smears are not necessary, so that threads and mucous strings need not be flattened out, but may be stained with the organisms in situ."

Auramin is a yellowish aniline dye, readily soluble in hot water and alcohol, of about the same price as methylene-blue, and can be obtained from Grubler or his English agents.

PAPPENHEIM'S METHOD.

Pappenheim,* after discussing the various stains used for showing the relation of the gonococci to the nuclei of the leucocytes, says :—" The ideal method for differentiating the cocci from the nuclei of the leucocytes is the methyl - green - pyrosin mixture of Pappenheim. The action of this staining mixture depends on the natural aversion of methyl-green for

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* Pappenheim : " Ueber Gonokokken farbung." *Centralb. f. Bakt.*, Ref. xxxiv. No. 1. 1903.

bacteria, and its exclusive predilection for the cell nuclei, whilst the pyronin is only a feebly-acting stain, and hence it does not stain the nuclei unless present in excess. The nuclei are stained bluish green ; the gonococci red."

I understand from his communication that it may be used as an after-stain for the gonococci, even when Gram's method is used. His staining solution can be obtained from Grubler or his agents, and costs about 1/- per 100 cc.

Diagnosis.—Having described the morphology, the position, and the staining reaction, the question naturally occurs : Can the gonococci be diagnosed with safety in film preparations alone ?

Foulerton,* as the result of his exhaustive enquiry into the subject, says : — " I believe myself that this recognition can, in the large majority of cases, be made a matter of absolute certainty by anyone who has had some little experience. The grouping of the diplococci in the pus cells, their long oval shape when they occur in this situation, and the comparative rapidity with which they are decolourised when treated by Gram's method, are together sufficiently character-istic to enable one to give a definite opinion should the gonococci be present.

" No opinion should be given if only isolated diplococci, free from the pus cells, are seen, even if they are decolourised by Gram's method, Nor should one be prepared to give an opinion unless a pus cell colony, containing at least four pairs of cocci, has been identified."

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* Foulerton : *loc. cit.*, p. 58.

The Bacillus of Soft Sore.

The infectivity of the soft chancre is now very conclusively proved to be due to the presence of a bacillus, discovered by Ducrey, and described later by Unna. It is spoken of as the strepto-bacillus of soft chancre (Ducrey), bacillus ulceris cancrosi (Kruse), and bacille du chancre mou by French writers.

These bacilli are short, compact rods with rounded ends, and measure about 1.5μ long, and 0.4μ broad.

They usually lie in groups of four, five, or eight, singly or in pairs, and are most frequently found in the intracellular spaces, but also occur in the protoplasm of the pus cells.

Babes[*] states that the arrangement of Ducrey's bacilli in short chains, or in parallel rows, in the homogeneous background, is especially characteristic. Unna found that in sections the bacilli were arranged in chains.

Macé[†] states that the bacilli, which are sometimes slightly constricted in the centre, occur singly, or more frequently joined in rows of two, three, four, or more elements, and sometimes, though rarely, form chains consisting of twenty to a hundred bacilli— hence the term strepto-bacilli given to them. At other times they are found in little masses.

In some cases the bacilli are abundant; in others rare. They are usually associated with other organisms, and are rarely found alone.

Bacilli arranged in chains, a characteristic on which Nicolle especially insisted, were not noticed by

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[*] Babes: " Der weiche Schanker." Handbuch der pathogenen Mikro-organismen. 1903. Vol. iii., page 430.
[†] Macé: Traité de Bactériologie. Paris, 1901, p. 881.

Ducrey, owing, as Wurtz[†] points out, to faulty technique, as the pressure of the pus between two cover-glasses causes the bacilli, which are originally in chains, to become dissociated.

They are easily stained by the analine dyes, but are quickly decolourised by alcohol and acids, and do not stain by Gram's method.

The extremities of the bacilli often stain deeply, whilst the central part remains clear.   (Macé. Zeissl).

Audry[‖] says that in pus the form of the bacillus is somewhat variable, but in the majority of cases he found them swollen at their ends, with a clear space between.   Bacilli arranged in chains were not frequently seen, although he found distinct chains of bacilli in pus from a bubo.   In the latter cases, however, the swelling of the extremities, and the clear central space, were less marked or even absent.

"The bacilli are sometimes isolated, sometimes in groups ; I have never seen more than seven or eight grouped together, and that rarely.   They occur sometimes outside the pus corpuscles, frequently they are found in the interior of the latter, where they are generally arranged in groups.   The groups are only met with in cases in which the bacilli are very abundant, but as this is not always the case, a careful search must often be made."

He further remarks, "I ought to say at once that I attach no value to the examination of the secretion from the primary sore ; the only exception to this statement being when the ulcer attacks the skin outside the balano-preputial or vulvar region."

[†] Wurtz : Precis de Bactériologie.   Paris, 1897, p. 472.

[‖] Audry : *Gazette Hebdomadaire*, March 4, 1893.

PLATE I.

Fig. 1.—Tubercle Bacilli in sputum. Stained by Ziehl-Neelsen's method with carbol-fuchsin and methylene-blue.

Fig. 2.—Gonococci in pus cells, and a few lying in the extra-cellular space. Stained with eosine and methylene-blue.

Fig. 3.—Pneumococci (Fränkel's) in sputum. Stained with dilute carbol-fuchsin.

Fig. 4.—Pneumobacilli (Friedländer's) in sputum. Stained with dilute carbol-fuchsin.

Fig. 5.—Pus containing Streptococci, Staphylococci and Diplo-cocci tetragenus. Stained with eosine and methylene-blue.

Fig. 6.—Bacilli of Influenza in sputum. Stained with dilute carbol-fuchsin.

Fig. 7.—Diphtheria Bacilli from a culture. Stained with Löffler's methylene-blue.

Fig. 8.—Diphtheria Bacilli from a culture showing Neisser's granules. Stained by a modification of Neisser's method.

PLATE I.
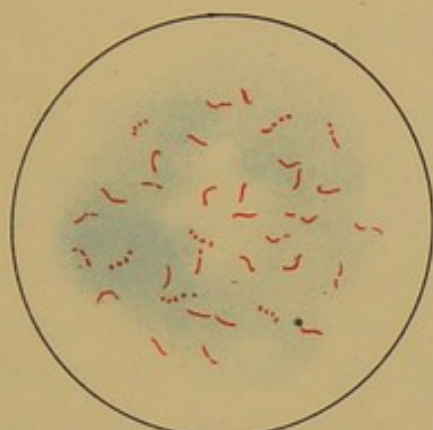
Fig 1.
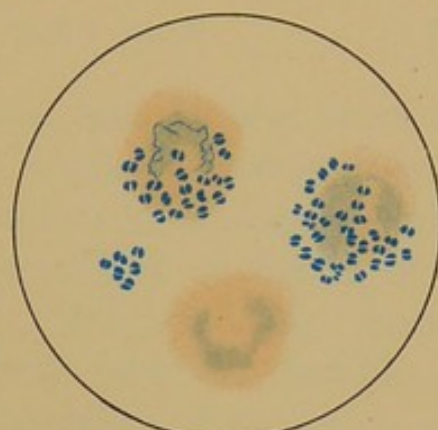
Fig 2.

Fig 3
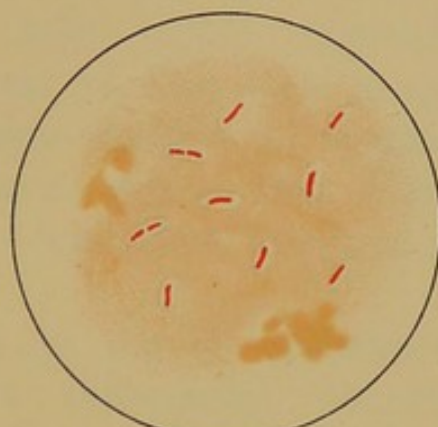
Fig 4.

Fig 5.

Fig 6.

Fig 7.

Fig 8.

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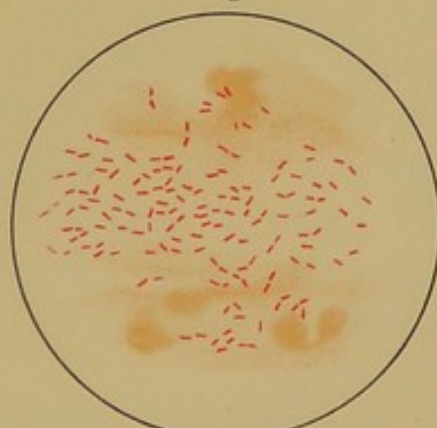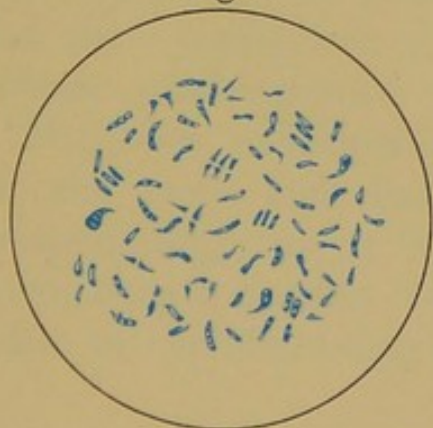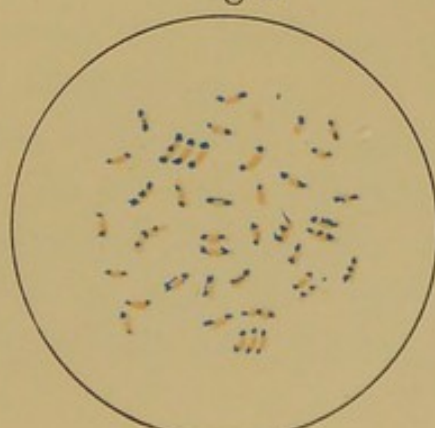
In the exudations of phimosis, and in the secretion from the urethra and vulva in women, Audry says he has constantly noticed the presence of a large number of bacteria, most frequently in the form of bacilli of all forms and dimensions, which stain well by Sahli's blue, and which are decolourised, like the bacillus of soft sore, by Gram's method, without there being the slightest indication of a chancre present. On the other hand, he has met with these somewhat characteristic bacilli in chancres of the skin outside the genital region, and has been enabled to diagnose the presence of a soft sore when the clinical appearance and position did not even suggest its nature.

In a private communication to me in reference to the possibility of making a differential diagnosis between a hard and a soft chancre, Audry says the examination for the bacillus of Ducrey in chancres of the genital region does not give good results, because there is no specific method of staining the bacillus, and because bacillary organisms are extremely frequent in all ulcerative lesions in this locality. Yet considerable help is obtained by finding the bacillus in soft chancres which occur outside the genital area, but these cases are infrequent.

He advises, as the best method of diagnosis, the inoculation of the patient with the pus from the sore, and the examination of the pus for the bacillus in the pustule resulting from such an inoculation.

My own experiences in the differential diagnosis of the soft and hard sore have been somewhat disappointing, as it is now generally conceded that Lustgarten's bacillus of syphilis is not the specific cause of syphilis, being probably merely the bacillus of

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smegma ; and, secondly, one has to remember that a
soft sore may, and not infrequently does, become a
hard chancre—*i.e.*, both the specific agent of syphilis
and the bacillus of Ducrey have been inoculated at
the same time, but that the former disease has a much
longer incubative period than the latter.

Macé,* however, states that "the search for this
organism may render great help in easily distinguishing
a soft sore from a syphilitic chancre, and this can be
done in secretions, or in sections of the excised
chancre."

With regard to the presence or absence of Ducrey's
bacillus in the bubo associated with a soft sore, it may
be said that the specific bacillus may be found in
about one-quarter of the cases examined, according
to H. Fournier.† In some the bacilli are virulent
and inoculable, whilst in a large number of cases the
pus of a bubo is found to be sterile in cultures,
and no organisms can be found on microscopic
examination.

PREPARATION OF AND STAINING FILMS.

In order to obtain film preparations the sore should
be washed with sterilised water, so as to remove,
as far as possible, extraneous organisms, and the
surface of the ulcer gently scraped with a probe or
knife, and the pus spread out in the usual way on the
surface of a slide, avoiding undue pressure.   The
films are dried in the air, fixed in the ordinary way,
and stained.

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* Macé : *loc. cit.*, page 881.

† Fournier :   "Le Traitement du Bubon Chancrelleux."   Thése,
Toulouse, 1897.

Audry says that Löffler's or Kühne's methylene-blue have given him negative results. He finds that Sahli's formula, which was used by Krefting, answered admirably. The formula for this is :— Borax, 16 ; saturated watery solution of methylene-blue, 24 ; water, 20 parts. Stain for half an hour (a shorter time will suffice if the preparation is warmed), wash, dry, and mount in balsam, or examine in oil. Preparations may also be stained with fuchsin, methyl violet, or gentian violet. A convenient stain, and one at hand, is Ziehl-Neelsen's fuchsin, diluted 1 in 10; stain from five to ten minutes.

Nicolle advises that the pus should be very carefully spread, so as not to break up the bacilli from their chain arrangement. He stains such films in gentian violet for about half a minute, washes and examines in water.

Diplococcus Pneumoniæ, or Fränkel's Pneumococcus.

The pneumococcus occurs in the form of a small oval coccus, one end of which is pointed, so as to give it the appearance of a grain of wheat (Talamon), the flame of a candle (Wurz), or the form of a lancet (Fränkel); hence the term diplococcus lanceolatus, which has been applied to it.

They are rarely isolated, but usually lie in pairs, or in short chains of four to six elements.

In the usual form—the diplococci—the pointed ends are directed towards, or away from each other. When united in the form of chains, each element lies with its long axis in that of the chain, and the chains

of diplococci are short, and never as long as those of the streptococcus pyogenes. (Plate I. Fig. 3.)

The actual size of the individual cocci varies considerably, but they usually measure about 1μ in their long diameter.

The cocci are surrounded by a capsule, which in some cases appears as an unstained halo; in others, according to the method of staining, as a structure which stains much less deeply with the basic aniline dyes than the organism itself.

The capsule is somewhat broader than the body of the coccus, and has a well defined outline. It may contain one, two or more cocci—*i.e.*, each element of the diplococci or streptococci is not supplied with a capsule to itself, although the latter may show signs of slight indentation around each pair in a chain of cocci.

According to Weichselbaum,* the capsule is associated with an active development of the pneumo-coccus, and it is not a sign of degeneration, as Pane considered. It is, he says, absent in cultures on artificial media, except in those few cases in which the medium is particularly suitable for its growth — *e.g.*, sterilised pneumonic sputum, or defibrinated blood. It is also absent in pneumococci, which only play a saprophytic part in the human organism, as well as in those which occur in the later stages of a disease.

Under unfavourable conditions of life atypical pneumococci occur in the form of single round cocci, or in long chains of such cocci, and in these a capsule is usually absent.

The pneumococcus of Fränkel retains Gram's stain

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* Weichselbaum: "Diplococcus pneumoniæ." Handbuch der path-ogenen Mikro-organismen. Vol. iii.. 1903, p. 198.

and this is of the greatest importance in distinguishing it from the pneumobacillus of Friedländer.

The diplococcus pneumoniæ can be found not infrequently under normal conditions in the mouth and nasal cavity. According to Weichselbaum, it is often met with in the larger and smaller bronchi in health, but not in the normal tissue of the lung.

It is by far the most frequent cause of croupous and catarrhal pneumonia, and may also be met with in pleurisy, endo- and peri-carditis, bronchitis, otitis, etc. Welsh[*] says that the pneumococcus can be found in every case of acute lobar pneumonia, and in most cases of bronchopneumonia, and in most of the mixed cases of pneumonia which are complicated with other organisms—*e.g.*, influenza, plague, typhoid, diphtheria, etc.

Of the serous membranes, the cerebro-spinal membranes are most frequently attacked, either secondarily to pneumonia, or as an independent condition.

Pleurisy, empyema, and pericarditis are not infrequently associated with the presence of the pneumococcus ; whilst in children otitis media and bronchopneumonia may be produced by these organisms.

In bronchopneumonia following influenza, the bacillus influenzæ may be found in enormous masses in the sputum, alone, or more frequently associated with the pneumococcus. In broncho-pneumonia of plague, diphtheria, typhoid, and in septic pneumonias, the respective causal organism may be found alone, or, more generally, in conjunction with the pneumococcus.

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[*] Welsh : " Bacteriology of Pneumonia." Encyclopædia Medica. Vol. ix., p. 414.

Pneumobacillus, or Friedländer's Capsule Bacillus of Pneumonia.

This organism occurs in the form of short or long rods with rounded ends, and is of a somewhat variable size. In sputum it may be a very short rod, almost like a coccus; generally, it is about 1μ long, but sometimes it exceeds 3μ. Its breadth is a little greater than that of the pneumococcus. (Plate I. Fig. 4.)

They occur singly, more frequently in pairs, sometimes in short chains. They are usually surrounded by a very distinct capsule, which is, however, usually absent in cultures, but reappears when the pneumobacillus is inoculated into animals.

They are not stained by Gram's method, and in this way are easily distinguished from the pneumococcus.

The pneumobacillus may be met with in health in the mucus from the mouth, nose and bronchi. Netter found it in the mouth of $4\frac{1}{2}\%$ of healthy individuals. It may, however, produce both local and general pathological conditions — e.g., stomatitis, rhinitis, and ulceration of the cornea, and may be associated with Löffler's bacillus in the production of a false membrane. It is also found in lobar and lobular pneumonia, pleurisy, pericarditis, etc., but not nearly as frequently as the pneumococcus. Weichselbaum only found the pneumobacillus nine times out of one hundred and twenty-nine cases of inflammation of the lungs; twice it was associated with the streptococcus pyogenes; and once with the diplococcus pneumoniæ. It is found more frequently in lobular than in lobar

pneumonia, and the pneumobacillus, although occasionally associated with the pneumococcus, is not generally regarded as the essential factor in typical lobar pneumonia.

METHOD OF THE EXAMINATION OF THE PNEUMOCOCCUS.

For practical clinical purposes, the pneumococcus will most usually be looked for in sputum, in pus, and in the exudations from an empyema or pleurisy.

Generally speaking, the pus resulting from pneumococci is thick, and rich in cellular elements. Even such an authority as Weichselbaum states that sputum can be examined just as well by film preparations as by cultures.

In the examination of sputum, the thicker, denser, gelatinous parts of the expectoration, as free as possible from saliva, are chosen, preferably from the rusty part of the sputum, and early in the course of the disease. Film preparations are made, allowed to dry, and fixed in the usual way by heat, or better by alcohol and ether, and then stained in one of the following ways.

The simplest method is to stain one film with carbol-fuchsin, in order to show the capsule, and another with Gram's method.

A few drops of Ziehl-Neelsen's carbol-fuchsin are filtered on to the film, and allowed to act for a minute or two, after which the stain is rapidly washed off with water, and the preparation slightly decolourised by immersing it for one second in alcohol, and then rinsing in water, after which the preparation may be dried and mounted. Simple as this method is, it

is the one recommended by Weichselbaum* for diagnostic purposes. By this means the background of the film is tinted, and the capsule appears either as a faint halo, or as a pale red zone round the lancet-shaped cocci.

Instead of alcohol, the film may be decolourised by placing it in weak acid acetic water for a few seconds.

Gram's method, as previously described, may be used, with weak watery solution of fuchsin as the after-stain.

Any of the ordinary aniline dyes will stain the pneumococci, but the more powerful stains are preferred, as they bring out the capsule better.

Double staining with eosine and methylene-blue, either separately or combined, as in Jenner's or Leishman's stain, show the relation of the organism to the pus cells, and give a very beautiful picture. The method is described under gonococci and the plague bacillus.

Although carbol fuchsin and Gram's stain are quite sufficient for diagnostic purposes, other methods, which aim at staining the capsule, may be mentioned.

RICHARD MUIR'S METHOD. †

1.—The film preparations are gently dried over the the flame of a spirit lamp, and a few drops of the mordant are filtered on to the film, and allowed to act for two minutes.

2.—The preparation is then thoroughly washed in water, then in methylated spirit, and again in water.

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* Weichselbaum : *loc. cit.*, p. 251.
† Encyclopædia Medica. Vol. iii., p. 505.

3.—Ziehl-Neelsen's carbol fuchsin is filtered over the surface, and the preparation gently warmed till steam rises, and allowed to stain for two minutes.

4.—The preparation is washed, and again treated with the mordant for two minutes, and again washed as before.

5—The film is then stained for two minutes in a saturated watery solution of methylene-blue, washed in water, differentiated in methylated spirit, dehydrated in absolute alcohol, and mounted in balsam.

The formula of the mordant is :—

Watery solution of Tannic Acid, 20% ... 2 parts.
Saturated watery solution of $HgCl_2$ ... 2 ,,
Saturated watery solution of Potash Alum 5 ,,

The pneumococci are stained a bright red; their capsules, even when the cocci themselves have degenerated, a blue colour.

Johne's Method of Capsule Staining. *

1.—Film preparations are fixed by passing through the flame three times in the usual way.

2.—Stain with 2% watery solution of methyl- or gentian-violet, with slight heat, for one to two minutes.

3.—Wash in water, and decolourise for ten seconds in a one or two per cent. acetic acid watery solution.

4.—Again wash in water, and examine in water, not in Canada balsam, as the refractive index of the latter prevents the capsules being seen. The cocci are stained a dark violet; the capsules a faint violet colour.

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* Encyklopädie der Mikroskopischen Technik. Wien, 1903, p. 900.

KLETT'S METHOD OF CAPSULE STAINING.*

1.—The film is dried in the air, and fixed by passing it through the flame.

2.—A watery-alcoholic solution of methylene-blue consisting of methylene-blue 1 part, alcohol 10 parts, and water 100 parts, is poured on, and the preparation heated over the flame till it just begins to boil. After cooling, it is thoroughly washed with water.

3.—Stain for five seconds with watery alcoholic solution of fuchsin in the same proportion as the above.

4.—Wash and examine in the usual way in oil or balsam. The cocci are stained a deep blue, their capsules faint red, and their margins a deep red colour.

FRIEDLÄNDER'S METHOD.†

1.—Fixed film preparations are treated for two minutes with a 1% solution of acetic acid.

2.—After washing and drying, they are stained with aniline water gentian violet, such as is used for Gram's Method, for a second.

3.—Wash in water, dry, and examine in oil or balsam.

RIBBERT'S METHOD.‖

1.—Stain for one minute in the following solution :—Distilled water, 100 ; alcohol, 50 ; acetic acid, 12·5 parts, saturated, when warmed, with dahlia.

2.—Wash in water, dry and examine in oil or balsam. The cocci are stained a deep blue, the capsules a faint blue colour.

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* Handbuch der pathogenen Mikro-organismen. Vol. i., p. 422.
† Handbuch der pathogenen. Mikro-organismen, Vol. i. p. 423.
‖ Macé. *Loc. cit*, p. 370.

SMITH'S METHOD.*

A useful, although somewhat long, process is the method devised by W. H. Smith for staining the pneumococci and influenza bacilli in sputum. It is useful for staining any organisms found in sputum.

Films of sputum are fixed in the usual way, and are then stained with aniline water gentian violet for a few seconds, gently warming the preparation until the staining fluid steams. Wash with Gram's solution of iodine for about 30 seconds, and again warm. Decolourise in 95% alcohol until the colour ceases to come out; wash in alcohol and ether four to six seconds; stain for one second with saturated watery solution of eosine, and wash away the surplus stain with Löffler's methylene-blue, and again dry. Decolourise with 95% alcohol for a few seconds, and clear with xylol, and mount in Canada balsam.

" The leucocytes, lymphocytes, as well as the red blood corpuscles, stain with eosine, whilst the cell nuclei take Löffler's methylene-blue. Bacteria positive to Gram stain deep violet or black, whilst those negative to Gram are blue. Bacteria with capsules have the latter tinted with eosine."

The pus from an empyema, or the sero-purulent effusion obtained by diagnostic puncture or aspiration of the pleura, can be examined and stained in film preparations just as sputum.

Fränkel's pneumococci are more commonly present than the streptococci in empyemas, even when these are unassociated with acute lobar pneumonia. (Welsh). This writer further remarks, that one frequently finds

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* *Journal of Royal Microscopic Society*, vol. vi., Dec. 1903, p. 772.

that the pneumococci have undergone degeneration to such an extent, that ordinary staining methods do not show their typical structure, and as in these cases cultures and inoculations may be unsuccessful, yet the organisms may be with certainty identified in film preparations especially stained to show the capsules of even these degenerated pneumococci. For this purpose he particularly recommends Muir's method as described above.

In cases of meningitis of pneumococcal origin, these specific organisms may be found in the sediment of cerebro-spinal fluid obtained by lumbar puncture. In this position, however, they have to be distinguished from the diplococcus intracellularis meningitidis of Weichselbaum.

In other complications of pneumonia, *e.g.*, arthritis, otitis, conjunctivitis, etc., film preparations may be made from the sediment of the aspirated fluid, or from the pus, and stained as sputum.

Diagnosis.—With regard to the recognition of the pneumococcus and the pneumobacillus, and the significance of their presence in sputum, I cannot do better than quote here the statement of such an authority as Weichselbaum,* who, in describing the bacteriological diagnosis of the diplococcus pneumoniæ, makes the following remarks :

" In cases of inflammation of the lung the sputum, freed as much as possible from saliva, will show not only the above described coccus, with or without its capsule, in its characteristic lancet shape, usually in pairs, but frequently even in pure cultures, and often in very large numbers. Of course these cocci retain

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* Weichselbaum : *loc cit.*, p. 252.

Gram's stain. In such a case at least we can with the greatest probability conclude that we have to deal with a pneumonia caused by the diplococcus pneumoniæ, a probability the greater if repeated examinations yield the same results.

"If we find in film preparations other cocci as well as the pneumococcus, this must be specially noted. If these are arranged in chains or heaps they may have originated from the saliva, and another preparation as free as possible from saliva should be made. If in the preparation freed from saliva the same condition is seen, and these are strepto- or staphylo-cocci, and retain Gram's stain, the case is one of lobar pneumonia with mixed or secondary infection, or one of lobular pneumonia.

"In cases of pneumonia caused by the streptococcus pyogenes alone, cocci in chains may only be met with, and these in very large numbers. This condition is of importance, as a pneumonia caused by the streptococcus pyogenes frequently shows certain variations in its course, and therefore the prognosis must be guarded with regard to the duration and the result.

"In cases of inflammation of the lungs induced by the *bacillus pnuemoniæ*, the sputum contains these bacilli with a distinct capsule, usually in very large numbers, so that their recognition causes no difficulty. Of course they must be decolourised by Gram's method. On finding these bacilli the possibility of the existence of chronic inflammation, 'sklerom,' of the air passages (larynx, trachea) must be thought of; but independently of the fact that the clinical examination will give indications which part is affected, the bacilli will be found in the sputum in large numbers in the course of a few days in cases of 'sklerom.' As a

pneumonia induced by the pneumo - bacillus frequently takes on an unfavourable course, the presence of these bacilli in sputum is of prognostic importance.

"Of considerable significance is the occurrence of influenza bacilli in the sputum, as these may be found in influenzal lobular pneumonia. It should be remembered in this condition the sputum is frequently not rusty, but frothy and purulent. Further, in pure influenzal lobular pneumonia. the influenza bacilli are usually, although not always, present in very large masses, and lie either free in small or large heaps, or within the pus corpuscles. They are to be recognised by their extraordinarily small size, as well as by the fact that they do not retain Gram's stain. Not infrequently, in addition to the influenza bacilli, pneumococci, strepto- and staphylo-cocci will be found which either come from the exudation of the pneumonia when the lung is the seat of a mixed or secondary infection, or from the bronchi.

"When the pneumococci predominate, the condition is probably one of lobular pneumonia produced by this organism, complicated by a secondary infection with the influenza bacillus.

"Inflammation of the lungs, occurring during an attack of plague or typhoid, is generally attended with sputum of a sanguinary nature. In plague, the plague bacilli are noticeable by their bipolar staining and their enormous numbers ; in typhoid fever short bacilli are present, which, like the plague bacilli, do not retain Gram's stain, but otherwise have nothing characteristic in their appearance, and for diagnostic purposes, cultures must be made.

"Cultures are also necessary when the micrococcus catarrhalis is suspected, and in all those cases in which

the above-mentioned bacteria cannot be recognised with certainty; when, for example, the pneumococci do not appear in their typical lancet form, or when their capsules are absent; when the streptococcus does not form distinct chains; when the influenza bacilli, or the plague bacilli, only appear in small numbers; or when the differential diagnosis is of the greatest practical importance.

"When the diplococcus pneumoniæ, the micrococcus catarrhalis, or the influenza bacillus is suspected, cultures on serum or blood agar should be made, in addition to those on the usual media."

The Bacillus of Influenza.

The influenza bacilli—one of the smallest of the bacilli—are tiny, straight rods with rounded ends. Their actual size is somewhat variable, their length being generally about two to three times their breadth. They are often described as about the thickness, but half the length, of the bacillus of mouse septicæmia. (Plate I. Fig. 6.)

Flügge* gives their average size as 0·2 to 0·3μ in width, and 0·5μ in length; Macé as 0·2 to 0·5μ in width, and two or three times as long.

Occasionally, as Pfeiffer points out, we discover longer forms in sputum and in cultures, and these are regarded as short filaments, whilst in old cultures very long filamentous forms — involution forms — are seen.

In some, but not in all, the central portion of the bacillus takes on the stain faintly, and appears like a

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* Flügge: Die Mikro-organismen. Leipzig, 1896. 3rd ed.

vacuole, whilst the terminal portions appear deeply stained, giving rise to the so-called polar staining.

As Pfeiffer says: "I am inclined to believe that some of the earlier observers also saw the bacillus described by me, but that, misled by their peculiar behaviour with regard to staining agents, they described them as diplo- or strepto-cocci."

Very commonly, the bacilli lie in pairs, end to end, resembling somewhat Fränkel's diplococcus of pneumonia, but differing from these by their much smaller size, their faint staining reaction, their shape, their not staining by Gram's method, and the absence of a capsule. In cases of sputum, in which the two organisms are found together, the difference in their appearance in a film preparation is very decided.

Threads of bacilli, arranged as strepto-bacilli, are, according to Flügge, seldom seen, but Pfeiffer, however, remarks that three or four bacilli, arranged in the form of a chain, are often met with.

In sputum the influenza bacilli occur in little masses made up of bacilli lying singly or in pairs, or in streaks between the pus cells.

During the febrile stage of the disease the bacilli may be found in enormous numbers in the sputum, and often in uncomplicated cases in almost pure cultures. At this time they are generally found in little masses, or in long trains in the mucus background of the preparation.

The pus cells during the height of the disease contain very few bacilli, but as the disease proceeds the number of organisms lying free in the mucus substance of the sputum diminishes, whilst those in the pus cells increase till the protoplasm of the latter appears full of these tiny bacilli; they do not, however, invade the nucleus,

When the acute stage is over and whilst the patient is convalescing, almost all the bacilli are found in the pus cells, and they now begin to show signs of disintegration, resist the action of stains, and can no longer be cultivated or easily recognised under the microscope. (Finkler).*

In those cases of influenza in which recovery is very delayed, Pfeiffer, Beck and Kruse have found bacilli—sometimes in very large numbers—in the expectoration, for weeks and months after the illness. Finkler records a case of influenza and bronchitis, in which the bacilli were detected for a period extending over three months, and Kruse found them four months after the commencement of the disease. Sometimes the bacilli were absent for a short time, only to return later. In cases of phthisis or chronic bronchitis, Finkler has pointed out that the influenza bacilli may remain apparently dormant, and these patients may, therefore, become centres of infection.

Notwithstanding the statement of Canon, Klein and others that the bacilli influenzæ may be found in the blood of patients suffering from the disease, the majority of observers, including Pfeiffer, Kruse, Goldscheider, etc., are emphatically of the opinion that the bacilli are not to be detected in the blood, and hence the general symptoms produced by the presence of the bacilli in the respiratory tract must be due to the toxic products of the bacilli — i.e., a sapræmia, not a septicæmia. This is known to be the case in many other conditions than influenza, especially in tetanus and diphtheria, in which the general symptoms are almost entirely due to the absorption of toxines.

* Finkler. Article on "Influenza" in 19th Century of Medicine. Vol. xv. (One of the best articles on Influenza in the English language.)

METHOD OF EXAMINATION.

The influenza bacilli are to be looked for especially in the sputum. This is often somewhat characteristic, generally being of a greenish-yellow or greyish-yellow colour. Sometimes it has a very yellow colour. It is viscid and adherent, particularly in the early stages. Finkler states that "especially noteworthy is the fact that the bronchial secretion soon becomes purulent in the presence of the influenza bacilli, so that rapidly occurring purulence of the expectoration, in a case of acutely progressive bronchitis, offers strong presumption that there is an influenzal infection."

The amount of expectoration varies considerably. In most cases it is small in amount, expectorated with difficulty, and very viscid. In others it is copious, and not nearly as tenacious, whilst in not a few cases there is no expectoration whatever.

In order to obtain good preparations, the sputum should be examined as soon after its expectoration as possible. The expectoration from the bronchi, that raised by a true cough, not by hawking, should be used for examination purposes, as the secretion from the naso-pharynx contains many other organisms from the adjacent cavities.

Secretions from the naso-pharynx will often contain streptococci and pneumococci, which, even under normal conditions, may occur in the posterior part of the throat and in the upper part of the bronchi, whilst the secretion from the deeper parts of the bronchi is, under normal conditions, free from bacteria. (Beck.)*

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* Beck : "Influenza." Handbuch der path. Mikro-organismen. 1903, vol, iii., p. 366.

The morning expectoration is generally the best, and to obviate as much as possible its admixture with extraneous bacteria in its passage through the mouth, it is well to advise the patient to rinse out the mouth two or three times with hot water before expectorating. The expectoration, received in a clean vessel—a Petri dish answers excellently—should be spread out, and by means of a dark background, the denser purulent yellowish-green matter, which often occurs in little round masses, can be picked out.

From these film preparations are made, allowed to dry in the air, fixed over the flame of a spirit lamp, or by immersion in alcohol and ether, and then stained.

Influenza bacilli are best stained, as Pfeiffer pointed out, with Ziehl-Neelsen's carbol-fuchsin, diluted about one in ten with water. Stain for about five to ten minutes. By this means the protoplasm of the cells, as well as the mucus background, are stained a pale red, and the nuclei of the pus cells, and the tiny influenza bacilli, a bright red colour. Other bacteria present are, according to Beck, as a rule stained a less intense red than the influenza bacilli.

Löffler's methylene - blue — stain for about two minutes—gives a less distinctive but pretty stain ; but the contrast between the influenza bacilli and the accompanying bacteria is, as Beck points out, not so pronounced, and, according to this observer, the influenza bacilli appear to be thicker when stained with the blue than when stained red with the fuchsin.

A point of some importance is that the influenza bacilli do not readily stain with the ordinary aniline dyes, so that if in a film preparation made and stained for the tubercle bacilli, and counter-stained with weak

methylene-blue, numerous very small, faintly stained bacilli are seen, much paler in colour than the other cocci, etc., present, I always suspect these to be influenza bacilli, and stain another film with dilute fuchsin.

They are not stained by Gram's method. Mallory and Wright describe Smith's modification of Gram's stain, as adapted especially for the differentiation in sputum of the pneumococcus and influenza bacilli. I have described this under the pneumococcus. (Page 139).

Cultures of the influenza bacilli are made by inoculating the surface of blood agar, prepared as follows, with influenzal sputum:—The lobe of the ear is carefully washed with soap and water, then with 1 in 1,000 solution $HgCl_2$, and finally with ether. It is then pricked with a sterilised needle, and the exuding drop of blood, caught on a sterilised platinum loop, is spread over the surface of the agar. The tube should be placed in the incubator, and its contents should remain sterile, and it is then ready for use. The opaque, greenish - yellow nodules picked out of the sputum are washed two or three times in sterilised water, and further dissociated with needles, and some of this material is spread over the surface of the blood agar.

After incubating for twenty-four hours at a temperature of 37°C, if the surface of the blood agar be roughly examined, it is quite possible nothing may be seen; but if examined carefully, in a good light, with the aid of a lens, minute transparent droplets will be seen. These colonies, which seldom exceed the size of a pin's head, are colourless, and possess a glassy transparent appearance, and resemble minute

droplets of water on the surface of the medium. A very characteristic feature, according to Finkler, is that the minute colonies always remain separate, and do not fuse together. Beck remarks that the growth is better, and appears quicker, when pigeon's blood has been used, instead of human blood.

Diagnosis. — I have often obtained the greatest assistance in diagnosis from a microscopic examination of the sputum in doubtful cases, or in cases in which, especially during an epidemic of influenza, the temperature of a chronic phthisical patient goes up without apparent cause.

Given a specimen of sputum, in which very minute bacilli are present in almost pure cultures, lying in heaps or in trains, often in pairs, which stain faintly with the ordinary aniline dyes, which occasionally show polar staining—(this is, in my experience, by no means constantly found) — and which do not take Gram's stain, then, I think, the presence of influenza bacilli may be diagnosed with great probability.

But in specimens of secretion from the nose or throat, or even in sputum which has not been examined for some time after its expectoration, it may be difficult, if not impossible, to say definitely whether some of the bacilli present are those of influenza or not.

With regard to the practicability of making a diagnosis from the microscopic examination alone, Macé[*] says :—" The diagnosis can be perfectly established by a microscopic examination of the sputum alone."

Finkler, of Bonn, in his excellent article on

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[*] Macé : Traité de Bactériologie. Paris, 1901, p. 842.

Influenza, makes the following statement :—" Does the microscopical finding alone suffice for the demonstration of the influenza bacilli?   Under certain conditions, this question may be answered affirmatively.   Anyone who has occupied himself largely with these bacilli will rarely err when he sees them in a sputum preparation.   He is perfectly justified in speaking of a typical influenza sputum, and there are many cases in which the clinical manifestations correspond to true influenza, and the bacilli are found in the sputum in pure culture.   In the expectoration, the bacilli often have a characteristic arrangement, lying together in long trains, so that they have been compared to schools of fish, and often they are present in such enormous masses that they cover the entire field of vision.   It is most desirable, however, to establish the identity of the bacilli, if every doubt is to be excluded, for this method leads to the certain demonstration of the bacilli, often more rapidly than the microscopical examination.

" The mode of cultivation here described proves sufficient for the diagnosis in many cases in which the microscope fails.   The latter may be the case either when the bacilli are too sparse — perhaps partly degenerated and enclosed in cells—or when other morphologically similar bacteria are present, as in the secretion from the mouth and neighbouring cavities."

The Meningococcus.

MICROCOCCUS MENINGITIDIS CEREBRO - SPINALIS, OR
DIPLOCOCCUS INTRACELLULARIS MENINGITIDIS.
(WEICHSELBAUM.)

In epidemic cerebro-spinal meningitis the following organisms have been met with :—

The diplococcus cerebro-spinalis ; diplococcus pneumoniæ ; strepto- and staphylo-coccus pyogenes ; and the bacillus of influenza, pneumonia, typhoid, coli communis, glanders and plague. By far the most frequent are the first two.

Marchal, quoted by Weichselbaum,* found that in sporadic and epidemic cerebro-spinal meningitis together, the meningococcus was present in 69·2%, the pneumococcus in 20·8%, and other bacteria in 10% of the cases.

In primary sporadic cerebro-spinal meningitis alone, the meningococcus in 50·5%, the pneumococcus in over 42%, and other bacteria in over 7% of cases. In epidemic cerebro-spinal meningitis alone, he found the meningococcus in 73·4 %, the diplococcus pneumoniæ in over 16%, and other bacteria in 10·5% of cases.

The meningococcus resembles very much the gonococcus. It occurs, as a rule, as a diplo-, sometimes as a tetra-coccus, and is generally arranged in small or large heaps. The cocci are somewhat round or oval, having the surfaces which are in contact flattened, resembling very much a coffee-bean. They vary very much in size, which, to some extent, depends on the method of staining adopted.

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* Weichselbaum : " Meningokokken." Handbuch der path. Mikro-organismen. 1903, vol. iii., p. 285.

Most frequently they are found, like the gonococci, inside the protoplasm of the cell, especially the pus corpuscle, although occasionally they occur free in the background of the preparation, sometimes in large numbers, at other times sparsely.

They are easily stained with the usual dyes, but never, according to Weichselbaum, retain Gram's stain, whether in exudations, cultures, or sections.

By means of a lumbar puncture, we are now able, as Osler* points out, to make in many cases a prompt decision of the existence of meningitis and its form.

Usually, the fluid so obtained is turbid; sometimes, but rarely, it is clear; whilst at other times it is clear at one puncture, and turbid at the next. The amount of fluid varies from a few drops to 130 cc., and often, according to Osler, there is a relation between the turbidity of the fluid and the severity of the symptoms.

The same observer remarks that in acute cases, the diplococci are usually present in large numbers in film preparations, whilst the later the disease the less likelihood is there of finding them, although he has met with them as late as the seventh week.

The fluid is collected in a sterilised test tube, or in a conical sediment glass, and film preparations made from the sediment. In cases in which the fluid is very clear, it may be necessary to centrifugalise it. Such preparations are fixed, and stained with any of the usual dyes, and also with Gram's stain.

Eosine and methylene-blue, one after the other, or more easily, with Jenner's stain, as described under the gonococci, show the bean-shaped diplococci in their intracellular position.

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* Osler: Encyclopædia Medica. Edin., 1901, vol. vii., p. 500.

Weichselbaum* remarks that if many pus corpuscles are found in the fluid, this points to meningitis, but the absence of these cells does not exclude this condition with certainty. The criterion is the presence of a specific organism.

He suggests that film preparations made from the sediment should be stained by Gram's method, and dilute watery solution of fuchsin used as the contrast stain.

" In this way all those organisms causing meningitis which retain Gram—*e.g.*, the diplococcus pneumoniæ, strepto- and staphylo-cocci pyogenes — are stained violet ; those which do not take Gram — *e.g.*, the diplococcus cerebro-spinalis, pneumo-bacillus, bacillus coli communis, typhi, influenzæ, and the bacillus of plague—are stained red.

" The differential diagnosis of the specific cause of the meningitis lies chiefly between the diplococcus pneumoniæ and the diplococcus cerebro - spinalis, and the latter usually lies inside the leucocytes, generally appears in the form of a diplo- or tetra-cocci with the opposed surface flattened, and sometimes shows inequality in size and intensity with which it is stained ; whilst the diplococcus pneumoniæ most frequently lies outside the cells, is somewhat roundish or oval in shape, possesses a distinct capsule, and occurs in pairs, or short, or even longish chains.

" The distinction of the latter from the diplococcus cerebro-spinalis can, in very many cases, be made with certainty in film preparations.

" When it is found in films stained by Gram that bacteria negative to Gram are present, other films

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* Weichselbaum : *loc. cit.*, p. 300.

should be made and stained with the most appropriate stain.

"It not infrequently happens, owing to the number of bacteria found being so few, that it is necessary to examine several films with the greatest care before any can be met with, and in such cases, as well as in those in which any doubt exists as to the nature of the bacteria present, cultivations should be made."

The Bacillus of Diphtheria.

The Klebs-Löffler bacilli, or Löffler's bacilli, as they are termed, are slender, straight, or slightly bent rods, usually somewhat swollen at one or both extremities. Their size varies greatly, but on an average, according to Löffler, they are about the length, but double the thickness of the tubercle bacillus.

Escherich[*] distinguishes three forms, (*a*) wedge-shaped rods about 1·5 to 2μ long, and 0·5μ thick ; (*b*) long cylindrical rods, 3 to 4μ long, and 0·4 to 0·5μ thick, and (*c*) rods with clubbed swellings, especially found on serum, which may attain 6 to 8μ long. In the wedge-shaped and clubbed forms the thin ends are often long and drawn out to a point.

The short forms are more frequently parallel in arrangement, the long forms arranged more at an angle, or in rosettes.

The shape and size largely depend on the age of the cultures and the nature of the medium on which they are grown. (Plate I., Fig. 7 and 8).

According to Kruse[†] the young diphtheria bacilli

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[*] Lehmann and Neumann : Bacteriology : 1901, p. 390
[†] Flügge : Die Mikro-organismen, 3rd Ed., 1896.

occur in the form of a narrow blunted wedge, which assumes a delicate spindle form when about to divide. When division has taken place the bacilli have the appearance of two blunt wedges, touching at their broad ends. This position does not last long, as usually one of the number turns itself, so that at first it lies at a right angle forming the letter L, and later the letter V, and eventually is arranged parallel with its fellow. Continued division and re-arrangement bring about the characteristic palisade or parallel formation.

Young cultures are made up chiefly of wedge or spindle shaped forms, but sooner or later dumb-bell or club shaped forms are found. These are much larger than the younger rods, and show a difference in their reaction to stains. Whilst the younger forms take on the stain evenly, and "appear as a darkly stained unsegmented mass of protoplasm, covered by a well-developed sheath which is slightly drawn out at one end," the somewhat older, larger, and more or less dumb-bell shaped bacilli stain irregularly or in patches, and show in their substance more deeply stained segments, alternating with paler areas, so that the bacillus has a stippled or dotted appearance. The ends may appear swollen and deeply stained, or they may not infrequently be tapered off and faintly stained, suggesting the appearance of a sheath.

Sometimes, especially in older cultures, one end of the bacillus is swollen or clubbed, the other pointed, giving rise to the pear or club form.

"We may regard, with a certain right, the club form as the fundamental form of the growth of the bacillus of diphtheria. This form is met with in smear preparations as well as sections of the diphtheritic

membrane.   In addition to it we find dumb-bell, spindle and lancet shaped bacilli, but the club form is generally the chief type, and other forms may develop from it.   It usually happens that very few bacilli are seen in the diphtheritic membrane, so that it needs, under certain circumstances, considerable practice to make a diagnosis from smear preparations alone. Not infrequently we find cocci and bacilli mixed with what we recognise as the characteristic club and dumb-bell forms of the diphtheria bacilli.   In other cases we find in smear preparations made from quite fresh diphtheritic membrane, the characteristic rods almost alone, and the diagnosis is made without further difficulty.   In many cases only a probable diagnosis can be made from microscopic preparation of the membrane alone, and in these it will be necessary to resort to cultivations made on suitable media."   (Beck).*

The diphtheria bacilli are found towards the superficial layers of the membrane, where they may occur in almost pure culture, or on the surface of the false membrane, in which case they are usually mixed with other organisms.   Of the associated bacteria the streptococci pyogenes are the most common and important, although staphylo- and pneumo-cocci, etc., may be present.   The streptococci are probably answerable for many of the complications of diphtheria, e.g., inflammation or suppuration in the neck, hæmorrhagic conditions, etc., and microscopic examination of the membrane may give valuable indications for treatment ; for example, the use of antistreptococcic serum in addition to antidiphtheritic serum.

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* Beck : " Diphtherie." Handbuch der path. Mikroorganismen. 1903. Vol. ii., p. 766.

The diphtheria bacilli stain readily with the ordinary aniline dyes, but some of the latter are more suitable than others for bringing out the structure of the bacilli.

By the use of certain colouring reagents, *e.g.*, old methylene-blue solutions, granules may be seen lying generally at both ends, sometimes also in the centre of the bacilli, and these stain a reddish tint quite different from the general body of the organism.

These are spoken of as metachromatic or Babes-Ernst granules or bodies, after the name of the discoverers. They are most easily stained by Neisser's method, or its modification, and constitute one of the most important points in the diagnosis of the true Klebs-Löffler bacillus. The best method of demonstrating these, their appearance and importance, will be mentioned later. (Plate I., Fig. 8).

The diphtheria bacilli stain by Gram's method, but as Beck[*] points out, great care is required in using this stain, as the bacilli may be decolourised by too long immersion in the iodine solution, and the contrast stain should not be a powerful one. For the latter purpose Bismark brown answers very well.

Macé[†] also says that, although the bacilli are not decolourised by Gram, yet they are often only imperfectly stained, and advises that preparations should not be subjected to the action of alcohol for long, but should still have a greyish-blue tint.

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[*] Beck : *loc. cit.*, p. 774.
[†] Macé : Traité de Bactériologie, p. 578.

Pseudo=Diphtheria Bacilli.

These bacilli, first described by Löffler, and later by von Hoffmann-Wellenhof, and usually spoken of as Hoffmann's bacilli, are closely related to the true Klebs-Löffler bacillus.

They occur in various anginal conditions, diphtheritic and non-diphtheritic, and to a certain extent, in the throats of healthy individuals

They are shorter and thicker than the Klebs-Löffler bacilli, and usually the ends are pointed. Like the latter, they are often arranged in parallel formations, but show less tendency to the formation of segments or clubs, and are not virulent for guinea-pigs.

The growth on blood serum is practically indistinguishable from that of the true Klebs - Löffler bacillus.

When stained by Neisser's method, granules are very rarely seen in the pseudo-diphtheria bacilli.

The Xerosis Bacillus of Neisser, found in certain cases of conjunctivitis, is another organism resembling Löffler's bacillus.

It is a short rod, about four times as long as broad, but not infrequently forms long threads, the ends of which are often clubbed. Like the true diphtheria bacillus, it shows differentiation of its protoplasm into segments. Neisser's granules are only rarely found in these bacilli.

The Method of the Examination for the Diphtheria Bacillus.

The presence of the diphtheria bacillus can be determined in a certain number of cases by the

direct examination of film preparations made from the membrane itself, or from swabs taken from the throat. In many cases, however, it will be necessary to resort to cultures.

(A) *Direct Examination of the Membrane.*—If the reports of the numbers of cases, in which a certain diagnosis was made from smear preparations, be examined, we are struck with the small percentage of cases in which a successful result was obtained. This is, I think, due to two or three causes. In the majority of cases, the swab is taken by the medical attendant, and examined by the bacteriologist, and the former probably usually makes a general swab of the whole affected area. A certain amount of time elapses before the swab comes into the hands of the bacteriologist, and during the interval, the putrefactive organisms have multiplied, or the swab has become dry ; and, although cultures can usually be successfully made from it, smear film preparations are often unsatisfactory.

My experience justifies me in thinking that if the bacteriologist were in a position to take the swab or membrane himself, just from the part he knows is most suitable ; if, in addition, care be taken to avoid contamination with the saliva and mucus of the mouth, and if film preparations were made at once, a very large percentage of cases of diphtheria could be diagnosed by the direct microscopic examination alone. A positive result in such cases would settle the diagnosis ; a negative result would be of no use, and cultivations should without delay be made in the latter, and also preferably in the former case.

I have in a large percentage of cases been able to make a diagnosis directly from the membrane or swab

even whilst the patients were waiting, and this has afterwards been confirmed by cultures.

The important points to attend to are (i) to procure if possible a piece of the membrane itself, or a good swab made by well scraping the affected area, (ii) to avoid the loose mucus often present, (iii) to make thin film preparations from that as soon as possible, the earlier the better, and (iv) to use a distinctive staining method.

The film once made need not necessarily be stained or examined at once.

Most of the bacteriological institutes supply swabs, which generally consist of a sterilized test-tube containing a stiff piece of wire having non-antiseptic wool firmly wrapt round one end, and fitted with a cork at the other. They answer admirably, but, if one is not at hand, a test-tube sterilized by boiling water in it, and a piece of stick or wire provided with wool firmly fixed at one end, can be used. If these are not procurable, and to save another journey, a penholder may be substituted for the wire, and a clean bottle for the test-tube. Sometimes a pair of forceps will be necessary for taking hold of a piece of partially detached membrane.

If a piece of membrane is obtained, this should be teased up with needles, and then crushed between two slides, one of which will then have a fairly thin film. Should the membrane have become dry, it must be teased up with a drop of water.

In cases in which no actual membrane is detached, the swab itself should be rubbed over the surface of the slide; sometimes it is advisable to slightly warm the latter, so that the particles may at once adhere to the glass.

The films should be allowed to dry in the air thoroughly, and may then be fixed in the usual way by heat, or by immersion in alcohol and ether for a few minutes. I find, when fixed by slight heat, the films have a tendency to wash off, especially after staining with Neisser's acid methylene-blue, probably owing to the acetic acid present, and therefore, prefer to fix them in alcohol and ether for about one minute.

I find that of all methods of staining, the modification of Neisser's with iodine gives the most reliable results, both in membrane and in cultures; but I think it advisable also to stain another film with Löffler's methylene-blue.

Macé,* speaking of the value of the direct microscopic examination, says :—" We may in such preparations find small and characteristic masses of Löffler's bacilli in the membrane ; but if the examination be negative, it is not possible by this means alone to conclude the absence of the micro-organism in question, as this may only be present in the exudation in small numbers, or may be masked by other elements. In spite of this, the direct examination is always to be recommended, as it is the only means which can give exact results of the composition of the false membrane, and the nature of the different species of bacteria which are found in it, many of which only grow slowly, or not at all, in cultures.

" The use of Crouch's and Neisser's stains give better results than the ordinary methods of staining ; the former is specially recommended. The appearances previously mentioned, and particularly the presence of stained granules arranged as described, appear in these conditions to belong properly to Löffler's bacillus, and

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* Macé : *loc cit.*, p. 624.

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can differentiate it distinctly from other micro-organisms which are found here mixed with it.

"This is a valuable characteristic, and it enables us in many cases to make a diagnosis very rapidly.

"Yet it should be remembered that now and then true diphtheria bacilli do not show these polar bodies, whilst the pseudo-diphtheria bacilli may occasionally exhibit them. We must not, therefore, consider this peculiarity of staining as a truly specific character, and attribute to it an absolutely diagnostic value." He states, however, that cultures should be made in all doubtful or negative cases, and even in positive ones, so that the examination by the direct method ought to be regarded as a provisional one.

(B) *Method of making Cultures.*—Cultures should be made on Löffler's or ordinary blood serum.* On this medium the diphtheria bacilli develop rapidly, the other non-pathogenic organisms slowly.

The swab is gently rubbed over the surface of the serum, or a piece of membrane, separated from the throat, is well washed in sterile water, and a fragment of this, held with a sterilized pair of forceps or on a platinum loop, is streaked over the surface of the medium, avoiding as far as possible breaking up the serum. If the swab or membrane has become dry it may be moistened with a little sterilized water.

The tubes are then placed in the incubator and kept at a temperature of 37°C. They should be allowed to remain there about eighteen to twenty-four hours, although after twelve to fifteen hours, in some cases eight hours, colonies may have developed.

Beck states that at the end of twelve to fourteen hours, sometimes even after six to eight hours, small

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* These can be obtained from the Lister Institute, Chelsea Gardens, S.W.

transparent granule-like colonies may be seen with the naked eye. These rapidly increase in size, and, at the end of eighteen to twenty hours, reach the size of a pin's head, and become an opaque milky colour. These colonies, which are round and slightly raised, are more opaque in the centre than at the periphery.

With a sterilized platinum needle or loop, a scraping is taken from one of the colonies. If there is no visible growth, a scraping is taken from the whole surface; if there is a visible growth, from the most likely colony; and if the growth be confluent, from the upper half inch or so. (Hewlett).

A small drop of water is placed in the centre of a clean slide or cover-glass, and the contents of the needle mixed with it. Probably the one drop will be enough to spread three films. The usual mistake is to make the film preparations too thick. After allowing the preparation to dry, it is fixed in the usual way and stained by one of the following methods.

Staining.—Films made from the membrane or from cultures may be stained with Löffler's methylene-blue for two to three minutes, or even longer, as over-staining, according to Beck, does not readily occur. (Plate I. Fig. 7).

They are then washed in water and differentiated by placing them in weak acetic acid water (one or two drops of concentrated acetic acid to 50 or 60 cc. of water) for a second or so.

Again wash in water, dry in the air and examine in oil or balsam.

This is the method advised by Beck. Differentiation in weak acid is not generally considered necessary by most English writers, but I find it certainly renders the structure of the bacilli more evident,

Other of the more ordinarily used aniline staining solutions may be used, *e.g.*, carbol-fuchsin diluted one in ten with water; or aniline water- or carbol-gentian violet for Gram's method. By Gram's method we can exclude the non-resisting bacilli provided due care is taken in the process.

Beck points out that the diphtheria bacilli, when stained with Löffler's methylene-blue, appear smaller and finer than in the unstained condition, whilst they are thicker and larger when stained by dilute fuchsin or gentian violet.

NEISSER'S METHOD.

The metachromatic or polar bodies are by this means stained a blue or black colour, the body of bacilli brown. It is applicable to films made directly from the swabs as well as from cultures. Neisser advised that the cultures should be kept at 34° to 36°C. for nine to twenty-four hours.

He uses the two following solutions:—

A One gramme of methylene-blue (Grubler's) is dissolved in 20 cc. of alcohol (96%) and mixed with 950 cc. of distilled water, and 50 cc. of glacial acetic acid.

B Two grammes of vesuvin (Bismark brown) are dissolved in 1,000 cc. of boiling distilled water, and the solution filtered.

The fixed film preparations are stained in solution A for one to three seconds, then washed in distilled water, and next stained for three to five seconds in solution B. Again wash, dry, and examine in oil or mount in balsam.

When I first tried this method some years ago, I

found that, although occasionally successful, I more frequently met with failure. Writing on the subject, I said:—*"To anyone who has experienced the difficulty of double staining—say the leucocytes of the blood with eosine and methylene-blue—this is not surprising. I therefore regarded Neisser's method as most unreliable in cases in which one has to determine the presence or absence of the bacilli."

Even Hewlett,† who was the first to draw attention to Neisser's stain, says: "The staining solutions seem to keep well, but occasionally fail to act, so should be controlled on an undoubted diphtheria culture."

Probably my want of success was due—at any rate, it was, so I afterwards found out, in double staining with eosine and methylene-blue—to the use of tap instead of distilled water.

Kurth, according to Macé, pointed out that distilled water must be used in the two stages of washing, as the lime and magnesia salts of some of the ordinary tap water prevents the methylene-blue stain acting.

Mr. F. J. Tanner, the Borough Bacteriologist of Bournemouth, after experiencing similar failures, suggested modifying Neisser's method, by treating the films with a solution of iodine after staining with methylene-blue. The following is the method which we have found to give absolutely constant and reliable results.

i. Film preparations of cultures, or membrane, are fixed in the usual way.

ii. They are stained in Neisser's acid methylene-blue solution for about one minute.

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* Coles : "A modification of Neisser's stain." *Brit. Med. Journal*, 1899. Vol. i., p. 1,213.

† Hewlett : "Manual of Bacteriology," London, 1902. 2nd Edition, p. 219.

iii. Wash rapidly with, preferably, distilled water, and place in Gram's iodine solution or Weigert's modification, viz, iodine 1, pot. iodide 2, water 200, for about fifteen seconds.

iv. Again wash in water, and pour over the film either the vesuvin solution B; or, better, a weak solution of safranine, and after a second or two pour this off and place some fresh solution on.   Stain for about 15 seconds in all.   The safranine solution consists of 1% solution of safranine in equal parts of methylated spirit and water—diluted with five or six parts of distilled water.

Stained in this way the diphtheria bacilli appear as slender rods of a brownish colour when stained with vesuvin, or a faint red with safranine, containing generally a bluish-black granule at each end, with sometimes one in the centre.   According to Wurtz, a bacillus may even contain four granules of equal or unequal size. (Plate I.   Fig. 8).

The granules are usually round, rarely, in my experience, oval, and vary somewhat in size, but are usually as large, or somewhat larger, than the body of the bacillus in which they lie.   Sometimes the dot at one end is distinctly larger than that at the other end, and Wurtz states that by very high magnification an indication of the division of the central dot, when present, may sometimes be made out.

In smears made from the membrane or from swabs taken from the throat, certain forms of the leptothrix may have a slight resemblance to the diphtheria bacilli.   The ordinary form of leptothrix is never likely to be confused with the bacillus, but small pieces may appear as short rods with one or more

black dots in them. If a group of eight or ten bacilli about the same size are seen, and if most of these contain the typical dots, the case is almost certainly one of diphtheria.

Two cocci placed some distance apart may, if these happen to retain the blue they sometimes do, appear somewhat like a diphtheria bacillus, but if one remembers that the dark granules are never found free, and that cocci are separate, no difficulty will be experienced. When safranine is used instead of vesuvin, the bodies of the bacilli are stained much more distinctly, and its use will prevent any confusion.

The value of Neisser's Reaction. According to Neisser only the true, not the pseudo-diphtheria bacillus, stains in this characteristic way. Fränkel also confirms Neisser's statement.

Beck states that, although. Neisser's granules are present in many cases of diphtheria, they may be absent in some cultures, and they are not specific for the diphtheria group of bacilli. I found them in a culture of one of the pseudo-tubercle bacilli, viz., Moeller's grass ii. bacillus.

Löffler, Spronck and Kraus, at the Ninth International Congress of Hygiene in Madrid, speaking of the diagnostic advantage of Neisser's reaction, said "it was to be considered of great help as a means of differential diagnosis, but it was not sufficient alone to make an absolute diagnosis." (Beck.)*

Franke and Heinendorff found Neisser granules only in isolated examples of the Xerosis bacillus, but not in the pseudo-diphtheria bacilli. Simoni also

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* Beck: "Pseudodiphtheriebazillen." Handbuch der path Mikroorganismen. Vol. ii., p. 827.

maintains that the absence of these granules is characteristic of the pseudo-diphtheria bacillus.

Beck states that in order to absolutely distinguish the pseudo- from the true diphtheria bacillus, Neisser's method is not of great use, a certain diagnosis being only possible by means of animal experiments.

Hewlett[*] says the pseudo- and other bacilli do not give the diphtheritic reaction with Neisser's stain, and even if the pseudo-diphtheria bacillus be found, this author prefers to regard the case as one of diphtheria.

We may, from these somewhat diverse statements, conclude that (1) in the large majority of cases the true diphtheria bacillus gives the Neisser reaction, but this may occasionally be absent; (2) the pseudo-diphtheria bacillus only very rarely gives it, and the xerosis bacillus gives it rarely, but somewhat more frequently than the former.

It is interesting to note that cultures of diphtheria bacilli made at all temperatures up to 37°C. according to Macé, give the reaction, and I have found that it was not ordinarily necessary to keep them at a temperature of 35°C., as is usually advised.

Crouch's[†] method of staining the diphtheria bacilli.

Methyl-green ... 1% watery solution	5 parts.
Dahlia ... ,, ,,	1 part.
Water	4 parts.

Stain according to Macé for one second for cultures, two seconds for membranes, and after-stain with vesuvin or methylene-blue for two to three seconds. The body of the bacillus is stained brown or blue, according to the after-stain used, the granules ruby red.

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* Hewlett: *loc. cit.*, p. 220.
† Macé : *loc. cit.*, p. 578.

Beck, quoting Mitchell, stains for five to eight seconds in the one solution.

Roux Stain.* This is recommended by Beck in preference to Neisser's stain. It consists of the following :

SOLUTION A.			SOLUTION B.		
Dahlia violet	...	1.0	Methyl-green	...	1.0
Alcohol, 90%	...	10.	Alcohol, 90%	...	10.
Aq. destill. ad	...	100.	Aq. destill. ad	...	100.

This will be mixed in the proportion of one part of A and three parts of B. Stain for two minutes without heat. The mixture can be kept for a long time and does not form any precipitate. " The diphtheria bacilli especially in smear preparations made from the membrane, show the bacilli beautifully stained."

PIORKOWSKI'S METHOD.†

In this method Löffler's blood serum is used for preference for the culture medium. The cultures are incubated at a temperature of 35°C. for ten to twenty-four hours.

1. Stain film preparations with Löffler's alkaline methylene-blue, slightly warmed, for one to two minutes.

2. Decolourise very rapidly, one second, (according to Friedberger, five seconds), with 3% HCl alcohol.

3. Rinse in water, and counter-stain with $\frac{1}{2}$ % aqueous solution of eosine for 10 seconds.

4. Examine in cedar oil, or, with clearer results, in water.

The bodies of the bacilli are by this method stained

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* Beck : *loc. cit.*, p. 775.

† Schaüffler : *Medical Record, New York*, Dec. 6th, 1902. p. 895.

a pale red colour, whilst the polar or metachromatic bodies, are a dark blue-black colour.

SCHAUFFLER'S METHOD.[*]

The solutions for making the stain are—

Filtered solution of Löffler's methylene-blue 10 cc.
,,   ,,   Pyronin (Grubler) ... 1.5 cc.
3 % HCl Alcohol ...   . 0.5 cc.

The pyronin solution in the above consists of— pyronin, 0.5 grams ; distilled water, 10 cc. The HCl alcohol is absolute alcohol 97 cc., and 25 % HCl 3 cc.

1. Film preparations are fixed by passing through the flame three or four times.

2. Drop on the film enough solution to cover it, and let it stain one minute.

3. Wash thoroughly in running water ; mount in balsam or oil. " Thus stained the bodies of the bacilli are blue, the polar bodies bright ruby-red. The pseudo-bacilli are much smaller, and take on only the blue colour. The bacillus of Asiatic cholera, and of plague, when stained by this method, show granular bodies in their interior, but these bodies are not metachromatic, like those of the bacillus diphtheriæ." The writer points out that by this method only one solution is needed, that only for one minute, and no heat is necessary. He regards it as a rapid and reliable method of diagnosing the true diphtheria bacillus. Smears from fresh diphtheritic membrane, he says, show the characteristic staining, although not so clearly as in cultures.

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[*] Schauffler : *Medical Record, New York*, Dec. 6th, 1902. p. 895.

Bacteria in Pus.

Very many organisms are associated with the formation of pus, amongst which may be mentioned the staphylococcus pyogenes aureus, albus and citreus, the staphylococcus cereus albus, and flavus, the streptococci pyogenes, the bacillus pyocyaneus, coli communis, the micrococcus tetragenus, and others.

Of these the most important and most frequent are the staphylococcus pyogenes aureus and the streptococcus pyogenes.

It is obviously impossible by an examination of stained film preparation alone to determine the exact species of organism present in pus, but we can easily say whether we have to deal with a strepto- or staphylo-cocci infection, and this is a matter of no little importance in the diagnosis, the prognosis, and in the treatment with the appropriate serum.

Staphylococcus pyogenes aureus.

This is by far the most frequent of all the organisms met with in suppurative processes. It consists of a number of spherical cocci varying considerably in size, but generally measuring on an average 0.8μ in diameter. Neisser[*] gives this measurement as from 0.7 to 0.9μ, Kocher as from 0.5 to 1μ. (Plate I. Fig. 5.)

They usually occur in somewhat irregular groups or grape-like clusters, but may sometimes be met with

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[*] Neisser and Lipstein: "Die Staphylokokken:" Handbuch der path. Mikroorganismen. Vol. iii., p. 108.

singly or in pairs. In pus, according to Neisser, they are generally found in little heaps consisting, in the smallest of two or three, in the larger of nine to ten cocci. Sometimes they are found in pairs forming diplococci, or in fours forming tetracocci, and even in short chains, so that it is not always possible, as Neisser points out, to determine whether we are dealing with staphylococci alone, or with staphylo- and diplo-cocci, or even staphylo- and strepto-cocci. The most conclusive evidence of the presence of a staphylo-cocci is, he says, obtained by examining a fragment of a pure culture in bouillon in a hanging drop preparation.

When faintly stained, and if examined with a high power, it is possible sometimes to see signs of transverse division of an individual coccus.

They stain easily with the ordinary aniline dyes and retain Gram's stain.

The staphylococci have a very wide distribution, and can be found in milk, water, air, and—under normal conditions—upon the skin, especially of the head, in the mouth and vagina, and in the milk of healthy women.

In pathological conditions they may be found in all suppurative or inflammatory processes, either alone or in association with the streptococci, etc.

They are most commonly present in localised abscesses, carbuncles, boils, suppurative periostitis, osteomyelitis, endocarditis, and in abscesses generally.

Streptococcus Pyogenes.

This consists of a number of round, sometimes oval, cocci arranged in the form of a chain. The individual

cocci in pus have an average diameter of about 0·8 to 1 μ, but in cultures their size is more variable, and depends very largely on the age of the growth and the nature of the medium. (Plate I. Fig. 5.) The length of the chain varies in different species, but in pus it is usually made up of five to fifteen elements, whilst in sputum there may be more than fifty cocci in one chain, and this is also the case in growths on fluid media.

As many of the cocci in a chain divide at the same time, a chain of diplococci may sometimes be seen. The strepto-, like the staphylo-cocci, pyogenes are easily stained with aniline dyes, and most of them stain with Gram's method.

The streptococci are found in health in the mouth, nasal cavity, and vagina. In disease they are especially associated with spreading inflammation, suppuration, cellulitis, lymphangitis, erysipelas, pyæmia, puerperal fever, endocarditis, suppuration in serous membranes, and in joints, etc.

The streptococci play an exceedingly important part in phthisis, diphtheria and scarlet fever, and their recognition in the two former diseases is of the greatest importance in the prognosis and treatment. A case of diphtheria associated with streptococci treated with anti-diphtheritic serum alone, may improve rapidly with the additional use of anti-streptococcic serum.

The Micrococcus Tetragenus.

This consists of roundish or somewhat oval cocci lying generally in fours, sometimes in pairs. The cocci vary in size, but generally measure about 1 μ in

diameter. The tetrad may appear at first sight like a sarcina, but is distinguished by the fact that in the former the four cocci lie on the same plane and do not form cubes. (Plate I. Fig. 5).

It is a common inhabitant of the mouth where, according to Koch, it plays merely a saprophytic part. It is not infrequently met with in tubercular sputum, especially in that from a cavity, in abscesses of the lung, and in cases of suppuration in the region of the mouth and neck.

It retains Gram's stain.

Method of Examining Pus.—The pus should be collected with antiseptic precautions, and examined as soon as possible. Films are spread in the ordinary way, and, after fixing with heat or alcohol, are stained for a minute or so with any of the ordinary stains, of which Löffler's methylene-blue, carbol-thionin, or Gram's method, are specially suitable.

Staphylococci will be found in clusters, streptococci in chains, and micrococci tetragenus in fours. All three are stained by Gram, and lie chiefly in the intracellular space.

If diplococci are found lying almost entirely in the protoplasm of the pus corpuscles, they will probably be either the gonococci or the meningo-cocci, and will not be stained by Gram. For the differentiation of these, it will be necessary to consider the position from which the pus was obtained, as well as the morphological characters of these diplococci.

If diplococci are present in lancet form, surrounded with a capsule, lying outside the pus cells, and not stained by Gram, if, in addition, the pus is from an

empyema, or from sputum, these will probably be pneumococci.

The differentiation of many of the bacilli present will not be possible without the aid of cultures, or inoculations into animals.

Actinomycosis may be suspected if small yellowish granules are seen with the naked eye, and if these, on microscopic examination, are seen to consist of filaments and clubs, arranged radially, the diagnosis is practically assured.

The Bacillus of Plague.

The diagnosis of a case of plague is only made with absolute certainty by a full examination, consisting of microscopic preparations, cultures, and experimental inoculations by a skilled bacteriologist, and it may be therefore deemed somewhat out of place to consider the subject here.

But in outbreaks of epidemics in places in which plague is known to commonly occur, the clinical features, supplemented by a microscopic examination, may make it highly probable that we have to deal with a case of this disease.

In order to render the microscopic examination as serviceable and efficient as possible, I have ventured to describe the general appearance and methods of demonstrating the pest bacillus.

The plague bacilli, as found in pus or blood, are short plump oval rods with rounded ends,

They are somewhat swollen in the centre, and this part stains faintly or may be unstained, whilst the ends are deeply stained, giving the so-called " polar staining." (Plate II. Fig. 11). Usually they are found amongst the cells, sometimes singly, sometimes in pairs, forming diplobacilli. Long chains or threads of the bacilli are seldom seen in the tissues, although common in cultures.

Their size is variable. Albrecht and Ghon, as a result of a large number of measurements, give their averege length as 1·5 to 1·75 μ or even more, and their breath as 0·5 to 0·7 μ.

Although the oval bipolar stained form of the bacillus is of most importance for diagnostic purposes, yet the plague bacillus is characterised by great variations in its size and form.

Dieudonné* says that alterations in length are especially noticeable, and short oval cocci forms, as well as long rod or bacilli forms, are met with not only in cultures but also in the tissues, secretions and excretions, as well as in film preparations of the blood, spleen, and sputum of cases of plague. For diagnostic purposes he states it is important to know that in addition to these, numerous other variations occur in smear preparations, especially noticeable being pale irregular, swollen, or circular forms, frequently not unlike yeast cells, which either stain faintly or only in their margins.

These are regarded as involution or degeneration forms, and are to be found in the tissues of the dead, as well as in the living.

In pure cultures all these forms may be met with,

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* Dieudonné: " Pest," Handbuch der path. Mikroorganismen, Vol. ii., p. 478.

and, in addition, thread and chain forms. As a rule typical oval bacilli are less frequently met with in cultures than in fresh tissues.

Plague bacilli, in the form of chains, strepto-bacilli, may, as Rees† has described, occur in sputum.

Most authorities are now agreed that the pest bacillus, in some cases at least, has a capsule, but this is not seen in preparations stained in the ordinary way. Albrecht and Ghon use for the demonstration of it Pitfield's flagella stain, whilst Zettnow uses Löffler's method of staining flagella. They are said to be particularly well seen in film preparations made from the peritoneal fluid, from an infected mouse or guinea pig, although they may also be found, but less easily, in preparations made from cultures.

The German Plague Commission, after trying all the special stains for demonstrating flagella, have been unable to find that the pest bacillus possesses flagella, as Gordon stated. They were also not satisfied that this bacillus possesses spores.

Method of Examination.—The microscopic examination will usually consist in an investigation of the material obtained from a bubo, or from the œdematous tissue around it in a bubonic case, the blood from the lobe of the ear in the septicæmic form, and the sputum in suspicious cases of plague pneumonia.

If the bubo has already opened, films can be made from the discharge from it, but these will usually show a number of other organisms.

Where the bubo has not ruptured, a sterilised hypodermic needle should be plunged into the gland, and films made from the material thus obtained.

In mild cases in which the inflammation is more

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† Rees: *British Medical Journal*, Vol. ii. p. 1237. 1900.

chronic, and softening of the gland absent, it is advisable to make a small incision, with antiseptic precautions, and remove a small piece of the affected gland, from which smear preparations can be easily made.

Blood preparations, as described below, may be made from the blood from the lobe of the ear, the tip of the finger, or from the skin over an enlarged gland.

Staining.—The plague bacilli can be readily stained by any of the aniline dyes, of which Löffler's methylene-blue or carbol-thionine may be particularly mentioned. They do not stain by Gram. Kossel and Overbeck* have pointed out that a certain amount of knack is required to demonstrate clearly and constantly polar staining, and have found that when cover-glass preparations are fixed in the ordinary way by passing them through the flame, polar staining is often either entirely absent, or its presence is merely suggested. They say that the most suitable method for the demonstration of polar staining, is to fix the films in such a way, that any red blood corpuscles present will take on a greenish, not bluish, tint, when stained with methylene-blue.

This degree of fixation is best obtained, according to these observers, by placing the preparation of blood, or "tissue juice," in absolute alcohol for 25 minutes.

Soberheim says that this is more quickly attained by pouring a few drops of absolute alcohol over the air-dried preparation, and after letting it remain for a short time (about one minute), it is poured off, and the moistened film held over a flame till dry.

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* Encyklopädie der Mikroskopischen Technik: Berlin, p. 1,096.

It can then be stained with Löffler's alkaline solution of methylene-blue, or with dilute carbol-fuchsin for two to three minutes.

Kossel uses borax methylene-blue, which consists of methylene-blue, two parts in a 5% watery solution of borax, 100 parts—stain for half-a-minute. If the preparations are over-stained, they may be differentiated in alcohol or very dilute acetic acid, but this is not usually necessary.

Gotschlich stains preparations for one moment in undiluted Ziehl-Neelsen's carbol-fuchsin, and then washes them thoroughly in water.

Sometimes it is advisable to adopt Gaffky's method, especially with "tissue juice" or blood preparations, i.e., to treat the preparations with $\frac{1}{2}$% acetic acid watery solution for about half-a-minute, thoroughly wash in water, and then stain.

Very beautiful preparations can be obtained by using one of the modifications of Romanowsky's method of staining chromatin, which are so useful in the examination of malarial blood preparation. This means is particularly adapted for blood films, and shows very distinctly the polar staining of the bacilli.

There are two methods of making films of blood for the clinical diagnosis of the plague bacilli in the septicæmic form of the disease: the ordinary thin films, and specially prepared thick films. Both are used for the recognition of the malarial parasite, and I have described in detail their preparation and method of staining for this purpose elsewhere.*

The lobe of the ear, the tip of the finger, or the skin over the affected area, is first washed with soap

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* Coles: "The Blood, how to examine and diagnose its Diseases." Churchill, 1902. 2nd Edition.

and water, then with 1 in 1,000 Hg.Cl$_2$, and lastly
with alcohol and ether.   The skin is then pricked
with a triangular pointed needle, and the drop of
blood so obtained is allowed to touch one end of a
slide held between the finger and thumb of the
left hand.

Another slide held in the right hand is used to
spread this drop over the surface of the glass, by
passing it along the slide—holding the glass at an
angle of 45°, in a quick but steady movement from
left to right.   If the drop is not too large, a thin
uniform layer of blood is thus spread over the lower
slide.   This is rapidly dried by waving it to and fro
in the air.

Instead of a slide, the blood may be spread by
applying a shaft of a needle, held horizontally, to the
drop of blood, and by drawing this across the lower
slide, as Stephens has suggested, an excellent film
will be left.

The unfixed air-dried film may be conveniently
stained with Leishman's stain as follows: a few
drops of this solution are allowed to fall on the film,
and, by a slight movement of the slide, allowed to
spread over the whole surface of the film.

At the end of half-a-minute, double the quantity of
distilled water is added, and allowed to mix with the
alcoholic solution of the dye.   Intimate mixture is
hastened by rotating the slide from side to side.

The film is then allowed to stain for five to ten
minutes, when the stain is flooded off by adding distilled
water, gently, drop by drop, until the slide holds as
much as possible, when it is tilted, and the fluid
poured off, whilst drops of water are still falling on
it.   By washing in this manner all danger of a

sediment being left on the slide is avoided. It is advisable to allow a few drops of the distilled water to remain on the film a further one-half minute or so, when it is dried by waving to and fro, or better, by blowing air on it with a Politzer's bag. Heat must be avoided in drying the film. It is then examined in oil, or mounted permanently in acid-free Canada balsam, which can be obtained from Grubler.

These films have the advantage that the red blood corpuscles and leucocytes are well preserved, but possess the disadvantage that a large area must be examined before concluding that the bacilli are absent.

Ross's Method[*] of making films for the examination for the malarial parasites, has the advantage that a comparatively large amount of blood may be examined in one film.

A large drop of blood, equal to about 20 cubic millimetres, is received on a glass slide, and is slightly spread out over an area which can be covered by an ordinary cover-glass. It is then allowed to dry in the air, or warmed over a flame, without heating it as much as to fix the albumen.

When thoroughly dry, distilled water is carefully dropped on it till the whole film is covered, and allowed to remain there a minute or so, until it is seen that all the hæmoglobin has been dissolved out. If the preparation is to be stained with any of the ordinary aniline dyes, it must be fixed by immersion in alcohol; but if Leishman's stain is to be used, a few drops of it are placed on the still moist slide, and distilled water added as before. It is allowed to stain for five to ten minutes, when the stain is carefully

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[*] *Brit. Med. Journal*, Jan. 10th, 1903. p. 86.

flooded off with distilled water, and the preparation mounted as before.

Diagnosis.—The following communication from Dr. J. Bell, Superintendent, and E. A. R. Laing,[*] Assistant Superintendent, Government Civil Hospital, Hong Kong, which shows the advantage of thick blood films, and the practicability of making a diagnosis even without cultures, is of great interest :—

" The difficulty of detecting plague bacilli in the blood is so well known that we venture to bring to your notice a method which has hitherto given us very good results in all cases, both early and late.

" It will be found very useful to those in charge of general hospitals, where it is difficult to keep out plague cases, and where one is anxious to come to a diagnosis as early as possible.

" The blood films should be taken by Ross's method, making the film a little larger and thinner than for malaria ; decolourise and stain, when plague bacilli will be found. All our last cases have been diagnosed in this way, and the diagnosis confirmed at the infectious hospital."

As to the importance of polar staining from a differential diagnostic point of view, Dieudonné[†] says that this condition is seen in a number of other bacteria, especially the group of hæmorrhagic septi-cæmia, and in the bacillus of fowl cholera and swine fever particularly. These show polar staining as frequently as the pest bacillus, but can be distinguished by their smaller size, as seen in animal tissues.

Gotschlich once found polar staining of the bacilli of a doubtful typhoid culture, Kossel and Overbeck

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[*] *Lancet*, vol. i., 1903.   June 20, p. 1767.
[†] Dieudonné : *loc. cit.*, p. 481.

in bacilli coli, and in pseudo-tubercle bacilli in rodents, and Schilling also met with it in a bacillus pathogenic to rats. In the latter case, however, polar staining was either entirely absent, or its presence only suggested in smear preparations made from the tissues of inoculated animals, and the form of the bacillus is quite different — they are plumper, and generally longer than the bacillus of plague.

" Above all, these bacilli never occur in such large numbers in the internal organs as is generally the case with the plague bacilli.

" In any case, polar staining is an important aid to the differential diagnosis, but only when it is pronounced."

Neisser found that the plague bacilli showed double staining with his method for the demonstration of granules in the diphtheria bacillus, but the granules were quite small. Except the diphtheria bacilli, very few other organisms give this reaction, and it may prove, as Dieudonné remarks, of importance in the differential diagnosis. He, however, adds that Neisser only obtained this reaction in one race of pest bacilli.

" In many cases, a diagnosis may be made by microscopic examination alone, as in no other known condition than plague, do bacilli with the morphological characters of the plague bacillus, occur in the lymphatic glands. An examination of the blood will only give positive results in severe cases. And in every instance, on the occurrence of the first suspected case, every care to exclude possibility of doubt should be used before a positive opinion is given." (Muir and Ritchie.*)

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* Muir and Ritchie : Bacteriology. 3rd edition, p. 428.

Actinomycosis.

Actinomycosis, although rare, is just one of those diseases in which a microscopic examination of the pus, sputum, etc., is absolutely essential for its recognition, and as it is, in not a few cases, a curable disease, this is of vital importance.

Cultures are quite unnecessary for diagnostic purposes.

According to Sheridan Delépine[*] actinomycotic lesions in man have undoubtedly been repeatedly mistaken for chronic abscesses, tumours, chronic tubercular, or syphilitic lesions.

More than half the cases occur in the region of the head and neck; thus Leith found that out of 430 cases, actinomycosis attacked the head, neck and tongue in 55·75 %, the abdomen in 21·6 %, the lungs in 13·25 %, the skin in 2·5 %, whilst in the remaining 6·9 % of cases the primary seat was doubtful.

The commonest position in man is the lower jaw.

Actinomycosis attacking the lungs simulates bronchitis, broncho-pneumonia, pleurisy, and especially tuberculosis.

"These patients suffer from cough, fever, night-sweats, sometimes hæmoptysis, and their expectoration, which is muco-purulent or purulent, contains the ray fungus." (Wurtz).[†]

Godlee[‖] believes that actinomycotic disease of the chest is much more common than is generally

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[*] Sheridan Delépine : "Actinomycosis." Encyclopædia Medica, 1899. Vol. i., p. 69.

[†] Wurtz : Bactériologie. Paris, 1897. p. 483.

[‖] Fowler & Godlee. Diseases of the Lung : p. 453.

supposed, and has seen five cases in a little over a year.

He says the expectoration in a case of this disease may be simply mucus, or may be purulent, and it is sometimes stated to be rusty like that in pneumonia. Sometimes a patient will say that he has expectorated at one time a large quantity of offensive material.

He further remarks that a diagnosis may be made by simply finding, with a lens, or with the naked eye, the yellow particles which, though somewhat like crystals of iodoform, are paler in colour and globular, not flat.

They are sometimes present in great abundance, but are not often met with in very large numbers.

Godlee makes the following suggestive remark: "If on opening an abscess which is supposed to be of considerable size, either connected with the rib or pleural cavity, or spine, or kidney, or cæcum, the amount of pus is found to be unexpectedly small, and the hæmorrhage is very free, suspicion should be aroused; and, if after a few days of free drainage, it is found that insidious burrowing of the matter is taking place beneath the skin, a careful search should be made for the granules of actinomyces."

It must, however, be remembered that in a small number of cases, tubercle bacilli and actinomycosis may occur together in the lung of the same patient.

The character of the discharge, when an abscess has opened, varies somewhat in different positions.

It may be viscid, or lumpy, or like ordinary laudable pus, or it may be thin and yet tenacious.

The sputum may be fetid, viscid, and yellow, with small greenish masses in it. If left to stand it forms two layers, an upper mucus and a lower gluey layer, in which the fungus may be found.

Naked Eye characters of the fungus.—Whatever be the nature of the pus or sputum, it will often be seen to contain innumerable minute specks, which consist of the organism embedded in a layer of pus cells.

Wurtz[*] states that it is necessary to examine the pus microscopically as soon as possible, as "the fungus rapidly undergoes alteration, and the characteristic form of the parasite may not be found the next day." He quotes Guermonprez, who says, that it is often very difficult to make a diagnosis, even with the aid of a microscope.

If a little of the pus be spread out on a piece of glass, in a developing dish, or shaken up in a test tube with a little water, the actinomycotic grains will easily be seen, often in large numbers.

These vary in colour; usually, however, they are yellowish or brownish by reflected, and greenish by transmitted, light.

According to Boström[†] the young actinomycotic granules are transparent-grey, almost like mucus, especially in human beings; the older granules are opaque-white; whilst the still older ones are yellowish, yellowish-brown or yellowish-green, in colour, according as the threads or clubs are most prevalent. Those found in the intestinal canal are of a darker colour, owing to the action of the sulphur on them; whilst the fungi found in cattle are usually of a sulphur yellow tint.

They have been likened to grains of iodoform in the pus, but Muir and Ritchie[||] state that the distinctly

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[*] Wurtz: *loc. cit.*, p. 482.

[†] Schlegel: "Aktinomykose." Handbuch der path. Mikroorganismen. Vol. ii., p. 863.

[||] Muir and Ritchie: Manual of Bacteriology. 3rd edition, p. 270.

yellow colour is only occasionally found, "in fact in the human subject they occur much more frequently as small specks, of semi-transparent appearance, and of greenish-grey tint."

They are generally of a soft tallow-like consistence in man, but in cattle they are not infrequently hard and gritty, owing to the presence of calcareous deposits.

The size of these granules varies considerably. Sometimes they are only seen on microscopic examination, being about 0·01 to 0·2 mm., usually, however, they are quite visible to the naked eye, and attain a diameter of 0·75 mm.

They are described as usually being the size of a grain of sand, pin's head, or millet seed, seldom as large as a poppy seed or small pea. (Kitt).

Microscopic appearance.—A few of these grains are picked out of the discharge, or taken off the dressing with a needle, transferred to a slide, and covered with a cover-glass. Examined with a one-inch objective, they will appear as more or less spherical masses of a pale greyish-yellow or greenish-yellow tint, somewhat like a minute raspberry, with a coarsely granular surface. If the cover-glass be gently pressed down, the grains, which flatten out like specks of tallow, will now be seen to have separated into irregular, or wedge-shaped fragments of a faintly brown colour.

If the spherical, oblong, or reniform masses, of which the tufts are composed, be examined with a higher power—*e.g.*, ¹/₆ inch objective—they will appear as a rosette of clubs.

These simple means are generally sufficient for diagnostic purposes, and the character of the organism can easily be recognised with a magnification of 100 to 300 diameters.

Schlegel* says that stained film preparations have no advantage for diagnostic purposes, as the characteristic appearance may be lost by the crushing of the tufts, whilst the uninjured tufts may become too opaque when stained.

Stained preparations are, however, necessary for the examination of the finer structure of the fungus.

When examined under a higher power, either in preparations teased in water, or in three-quarter per cent. salt solution, and mounted in water or glycerine, or in stained film preparations, the following parts may be made out (Plate II. Fig. 9) :—

The centre of the fungus consists of interlacing filaments of 0·3 to 0·5 μ in diameter, forming a loose or dense network. These filaments are wavy long threads, often branched, which radiate towards the periphery. They usually stain quite regularly, but in some, especially the older colonies, cocci, spore-like or rod-like bodies may be seen.

Among the filaments are seen numerous cocci, measuring 0·5 μ in diameter, short bacilli-like rods, or short threads. These small, round cocci may be found in a filament, arranged something like streptococci, or free, in the centre of the young colonies. They have been spoken of as spores or conidia.

Probably the cocci and the short rod-forms are developed from a filament, or may themselves grow into longer threads.

Outside the central felt-work, composed of filaments and cocci, we have the thickened clubbed endings of the radiating filaments, as well as projecting filaments from the inner zone.

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* Schlegel : *loc. cit.*, p. 864.

The clubs are pear-shaped bodies, formed by a hyaline swelling of the sheath of the filament, which are arranged in a radial manner ; hence the term ray fungus given to the actinomycotic parasite. They have their thin end directed towards the centre, the thickened end pointing outwards.

The clubs vary in size, but measure, on an average, 10 to 60 μ in length, and up to 10 μ in breadth, and may show branching.

The relation of the clubs to the threads is well seen when preparations are stained with orange-rubin, the former being stained crimson, the threads blue. In the younger clubs the protoplasm of the thread may be seen in the interior of the club.

The proportion of clubs to threads varies in different species. In man, especially, in rapidly growing colonies, threads with comparatively few, or even no clubs may be found, although, as a rule, both clubs and threads are present, whilst in actino-mycosis in cattle, the clubs generally appear early, and are much more numerous, and the filaments, especially if calcification has begun, often become indistinct.

Muir and Ritchie* state that in the human subject the clubs are often fragile structures, which are easily broken down, and may even dissolve in water, and for this reason are well seen in the fresh condition, but may be indistinguishable in hardened preparations.

Around the whole actinomycotic follicle a layer of inflammatory cells, sometimes containing giant cells, may be seen, especially in sections.

Methods of Staining. — Film preparations, or sections, can be stained with various aniline dyes,

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* Muir and Ritchie : *loc. cit.*, 3rd edition, p. 273.

and of these Gram's method, or Weigert's modification, with or without special after-stains, gives most excellent results. For the preparation and staining of sections, I must refer the reader to the larger text-books of pathology and bacteriology.

In order to obtain film preparations, some of the small yellowish-greenish nodules, picked out from the pus, sputum, or from the scraping of the growth or granulation tissue, are placed in the centre of a slide, and by means of another slide, the material is spread out. Here it is of more importance to avoid breaking up the structure of the fungus than it is to obtain perfectly thin films.

The films, after drying in the air, are fixed by passing them over the flame in the usual way ; or, better, by immersion in alcohol, or alcohol and ether, for a few minutes.

Of the single dyes, staining in carbol-thionin for one to two minutes gives very good results.

Gram's method, with after-staining with eosine, is very suitable, as by this means the threads and cocci are coloured violet, the clubs pink. It must be remembered that in actinomycosis in man the threads retain Gram, the clubs do not ; but in cattle, the clubs, as well as the threads and cocci, retain Gram's stain.

Boström* stains cover-glass preparations with aniline water gentian violet, as in the first stage of Gram's method ; but, instead of then using the iodine solution, he decolourises the preparation in alcohol containing eosine or picric acid.

Film preparations, as well as sections, can also be

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* Encyklopädie der mikroskopischen Technik. 1903, p. 8.

stained with hæmatoxylin and eosine, or orange-rubin.

Crookshank* says that in Gram's method, with after-staining in orange rubin, we have a test that is as characteristic and useful as the stain for the tubercle bacilli; the filaments and cocci are stained blue, the clubs crimson, and the tissue elements pink.

By this method the prolongation of the blue threads into the clubs can be very clearly seen.

The streptothrix maduræ, the fungus found in madura foot or mycetoma, is closely allied to the actinomyces.

Anthrax.

Anthrax is found in man in the form of (1st) a cutaneous disease—the so-called malignant pustule, (2nd) an intestinal disease, and (3rd) as a pulmonary disease, *e.g.*, woolsorter's disease or anthracæmia.

It is more common in animals, and is conveyed to man from the carcases, skin, hair, and bodies of infected animals.

It is possible in many cases to diagnose the disease in man or animals by means of a microscopic examination of stained preparations alone, although it is advisable to confirm this by cultures and inoculations into animals.

In support of this statement, Bell† says, that in man "a positive diagnosis may be made directly by

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* Crookshank: Bacteriology and Infective Diseases. London, 1896. p. 433.
† Bell: "Anthrax." Allbutt's System of Medicine. Vol. ii., p. 535.

the microscopic examination of the fluid for the bacillus anthracis ; or indirectly by inoculating a white mouse with a drop of serum from a vesicle."

Hewlett* says that in animals it is often possible to make a diagnosis by means of cover-glass preparations, and "the stained preparations can be kept and produced in a court of law." He also remarks that cultivations on agar or gelatine may give positive results when the microscopic examination has been negative. The inoculation of a guinea pig or mouse should be made in cases of doubt, as this, he states, is by far the most certain method of diagnosis, a negative result being nearly as valuable as a positive one. He, however, draws attention to the fact that both cultivations and inoculations may fail to give positive results if the material be old or putrid.

DESCRIPTION OF THE BACILLUS ANTHRACIS.

If the blood or spleen juice of an animal, or under some conditions, of man, attacked with anthrax, be examined in the fresh and living condition, a large number of rod-like bacilli will be seen amongst the red blood corpuscles.

These are in length about the diameter of a red blood corpuscle, and measure, on an average, 4·5 to to 10 μ long, and 1 to 1·25 μ broad. They appear as homogeneous structureless cylindrical rods, with, in the fresh condition, rounded ends, and show no sign of movement.

They occur singly, or more usually joined together in twos or threes ; longer thread forms, though

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* Hewlett: Bacteriology. 2nd edition, p. 192.

PLATE II.

Fig. 9.—Actinomyces. The central portion consists of filaments stained blue, and the peripheral part shows the clubs stained pink. Stained by Gram's method and orange-rubin.

Fig. 10.—Spirillum of Relapsing fever; blood film preparation stained with eosine and methylene-blue.

Fig. 11.—Plague Bacilli in the pus from a bubo. Stained with eosine and methylene-blue.

Fig. 12.—Anthrax Bacilli in pus. Stained with eosine and methylene-blue.

Fig. 13.—Tricophyton microsporon, or small spored ringworm in Hair.

Fig. 14.—Tricophyton megalosporon, or large spored ringworm in Hair.

Fig. 15.—Achorion Schoenleinii, or Favus fungus, in Hair.

Fig. 16.—Microsporon furfur, the fungus of tinea versicolor.

[Figs. 13 to 16 after Malcolm Morris.]

PLATE II.
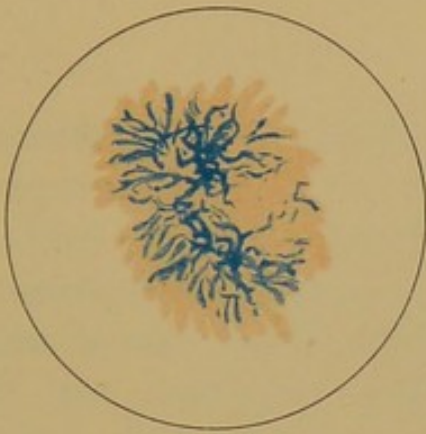
Fig 9.
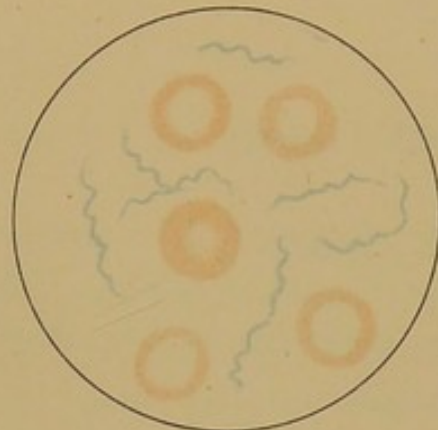
Fig 10.
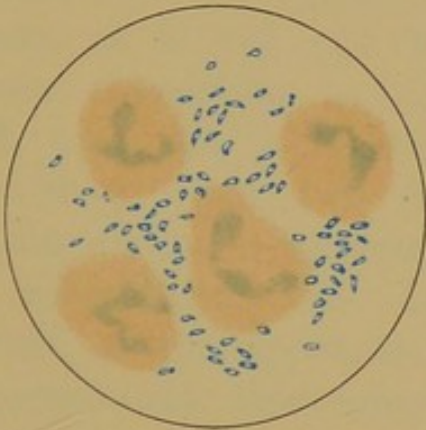
Fig 11.
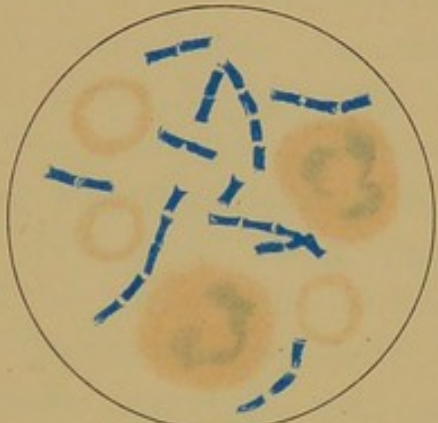
Fig 12.
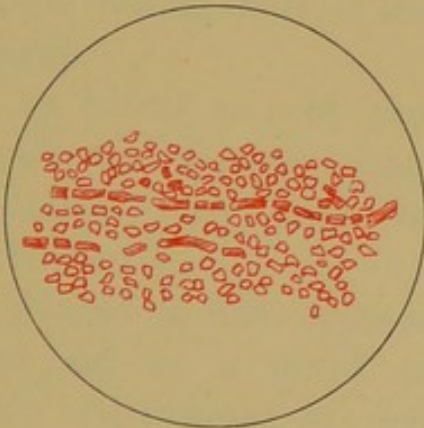
Fig 13.

Fig 14.

Fig 15.

Fig 16.

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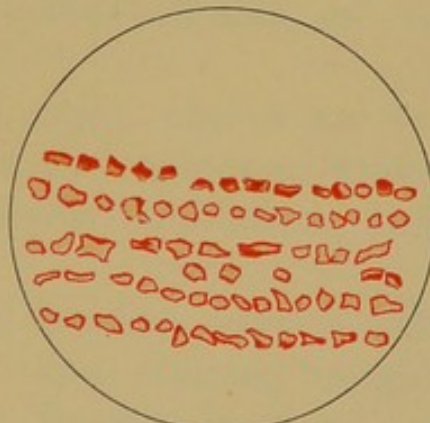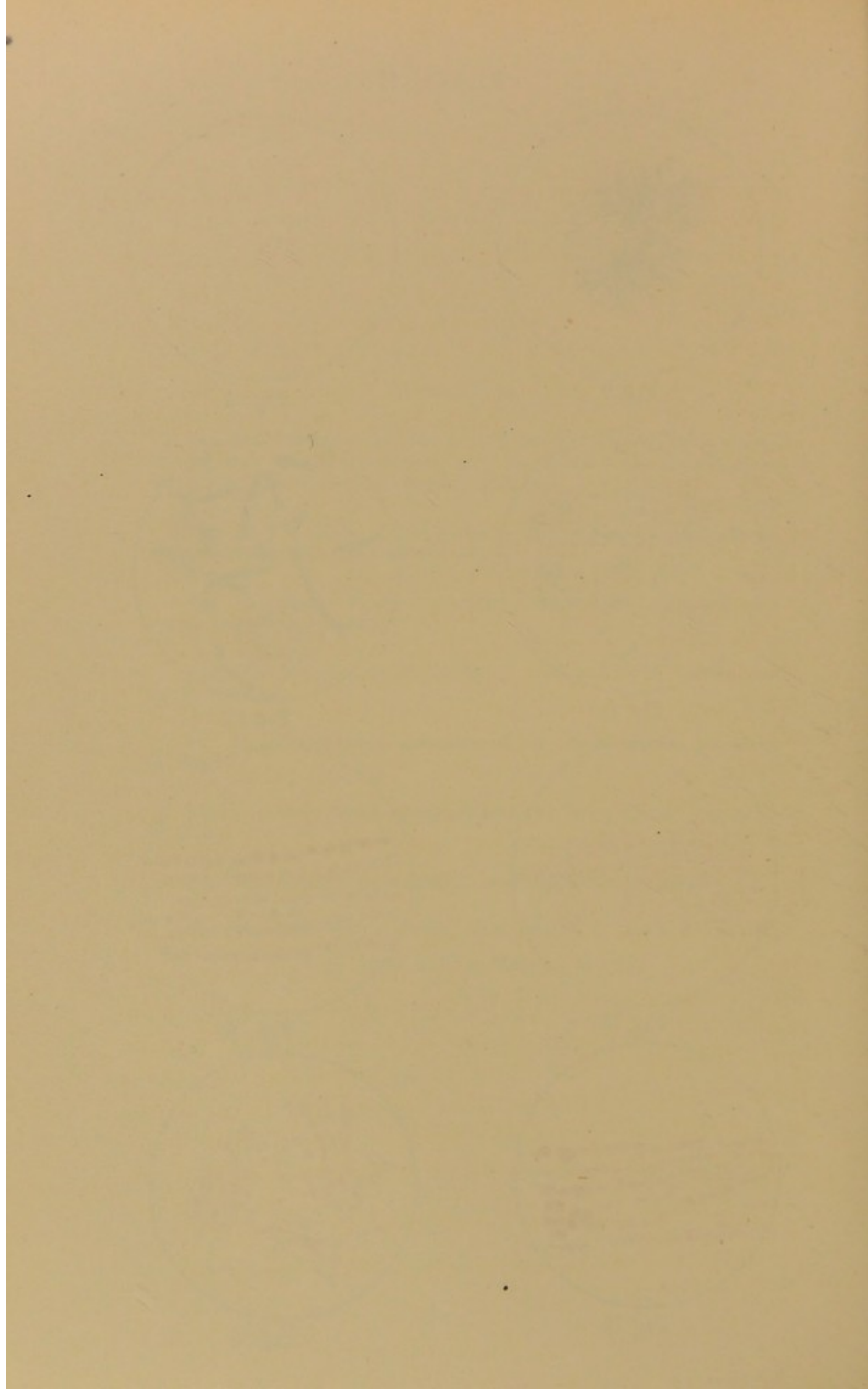
common in cultures, are rare in fresh blood preparations.

The anthrax bacillus, as seen in stained film preparations, appears somewhat different from that seen in the fresh condition. It stains easily with any of the ordinary aniline dyes, and retains Gram's stain. When examined under these conditions, the bacilli are no longer symmetrical rods with rounded ends, as is the rule in fresh preparations, but show some thickening at each end, which is abruptly cut off, and this is actually concave instead of convex, so that, when two bacilli lie together, there is a biconvex space between them. (Plate II. Fig. 12.)

Sobernheim* has compared a chain of anthrax bacilli to the appearance of a bamboo cane, with its characteristic thickenings at regular intervals, and says that, although this is not seen in all cases, yet it is often found, and is somewhat characteristic of these bacilli.

In blood, except perhaps in swine, chains are never made up of more than five or six segments, whilst in cultures they may be of almost unlimited length. (Hewlett.)

Macé† in his account of the bacillus, says :—"With the aid of a good objective and staining reagents, thin partitions, of which the length is more than double the breadth, are seen separating the bacilli. Often on the other hand, the bacilli making up a chain, appear separated from each other; between the two succeeding elements there occurs a clear space which is irregular in outline, due to the fact that the

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* Sobernheim : " Milzbrand." Handbuch der path. Mikro-organismen." Vol. ii., p. 9.

† Macé : Traité practique de Bactériologie. Paris, 1901. p. 483.

bacilli are not cut off at right angles, but terminate in a slightly sinuous line. This is a characteristic on which Koch strongly insists, and which he considers as characteristic of the bacillus anthracis. It enables us to distinguish this species from other bacteria, which are likely to be met with in the same conditions.

" It is only seen in fixed and stained preparations, and this suggests that it is due to the action of the reagents used ; but it loses none of its value from that. This characteristic is not ordinarily seen in preparations made from cultures."

Sometimes the bacilli, instead of being arranged in a straight line, lie at right angles one to another.

The bacillus anthracis is provided with a capsule. This is not present in all, and Serafini, who first noticed it, said that it was only to be found in fresh material, not in cultures. Other observers, whilst agreed that it is commonly met with in blood and tissues, have found it in some cultures.

For the demonstration of the capsule, any of the methods used for staining this structure described under the pneumococcus (page 136) may be used.

Under certain conditions, particularly the presence of oxygen, suitable temperature and culture media, the anthrax bacilli form spores. These are never found in the blood or tissues during life, and even some time after death, but are always met with in aerobic cultures, in which nearly every segment of the bacillus contains one. The anthrax spores are extremely resistant, and withstand, according to Koch, boiling for five minutes.

The fact that spore formation never occurs within the bodies of animals suffering from anthrax, unless

the bacilli are exposed to the oxygen of the air, is a matter of vital importance, as carcases of infected animals should not be opened, but buried entire with lime. The examination for diagnostic purposes is usually made from a small piece of the ear which is cut off.

Method of Examination.—In *man*, film preparations should be made from the fluid of the vesicles, from the scrapings of the incised or excised so-called pustule, in cases of the local form of the disease.

The blood should be examined, when the disease takes the form of a general infection, either by thin films, or by Ross's method of making thick films, as described under plague.

In *animals*, films should be made from the blood, or after death in cases in which the animal has been opened, from the juice of the spleen. J. McFadyean* states that:—" When an anthrax carcass is left unopened, the invasion by putrefactive bacteria is sometimes so complete within 24 hours, that not a single anthrax bacillus can be detected by microscopic examination in any of the organs in the chest or abdomen; but in the blood of the ears or feet, the anthrax bacilli may be recognisable on the third day after death.

" When an animal is unexpectedly found dead, and anthrax is suspected, if the carcass is already partially putrid, blood from an ear or a foot ought to be examined in preference to spleen pulp, or blood from one of the large veins of the body. At the present time, the material sent to the laboratory for examination in suspected cases of anthrax, is almost always

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* J. McFadyean: *Journal of the Royal Agricultural Society.* No. 18. 1894.

the spleen or part of it, and in a considerable proportion of cases, a positive opinion cannot be given because of putrefactive changes."

Films should be fixed by immersion for a few minutes in absolute alcohol, or alcohol and ether, but also, though not quite so satisfactorily, with heat.

The anthrax bacillus stains readily with any of the aniline dyes, and especially well with Gram's method.

Such films may then be stained with Löffler's methylene-blue, dilute carbol - fuchsin (1 in 10), or thionin, and also with Gram's method with eosine in the ordinary way.

Films may also be double-stained with eosine and methylene-blue, either separately or combined, as in Jenner's or Leishman's method, as described under gonococci and plague.

Films made from the malignant pustule generally show the characteristic bacilli in good numbers, but sometimes they are scanty ; whilst in blood films, anthrax bacilli are seldom seen in man, except in very severe cases shortly before death, and even then they may be absent. Possibly, the examination of a considerably larger drop of blood by Ross's method of making films, may render the diagnosis in this medium more successful.

Special Methods of Staining.—The *capsule* of the bacilli may be shown by using one of the special stains for this purpose — *e.g.*, Friedländer's, Johne's, Klett's, Muir's, etc., mentioned under the pneumo-coccus. (See page 136.)

Sobernheim states that Zettnow's modification of Romanowsky's eosine methylene-blue, stains the bacillus and its capsule exceedingly well. The body of the bacillus is stained blue, its contained chromatin red, and the capsule a pale reddish colour,

Probably, Leishman's modification of this stain, used as described under the plague bacillus (page 180), would give equally good results.

Spores in anthrax bacilli from cultures may be stained with Möller's* method as follows :—

1. The air - dried film is passed three times through the flame, or placed in absolute alcohol for two minutes.

2. Place in chloroform two minutes, to remove any fat.

3. Wash in water, and treat with 5 % chromic acid for two minutes.

4. Again wash, and drop filtered carbol-fuchsin on the film, and heat this over the flame, just as in tubercle bacillus staining, for one minute.

5. Wash, and decolourise in 5 % sulphuric acid.

6. Wash thoroughly, and counter-stain with a watery solution of methylene-blue.

7. Wash, dry, and mount in Canada balsam.

The spores stain red ; the body of the bacillus blue.

Sobernheim speaks most highly of A. Klein's method† of staining spores as being most reliable for the anthrax bacillus. The following is the procedure :—

1. Equal quantities, about 1 to 2 cc.m., of filtered carbol-fuchsin and of an emulsion of the spore-bearing bacilli in physiological salt solution, are mixed together in a watch glass, which is gently heated over a flame till steam rises, for about six minutes.

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* Encyklopädie der Mikroskopischen Technik., 1903, p. 901.
† Handbuch der path. Mikro-organismen. Vol. i., p. 424.

2. At the end of that time, a small drop of the mixture is taken out by means of a platinum loop, and spread in a thin layer over cover-glasses or slides. These are left to dry in the air, and then fixed, by passing them twice through the flame.

3. Decolourise in 1 % sulphuric acid for a few (one to two) seconds.

4. Wash in water.

5. Counter-stain in dilute methylene-blue solution, without heat, for three or four minutes.

Sobernheim suggests that if the above mixture is made in a test-tube, and heated over a gas flame, the staining is more rapid and intense, without in any way injuring the form of the bacilli.

The Differential Diagnosis. — The most probable organism met with in disease in man which might be mistaken for the anthrax bacillus, is the bacillus of malignant œdema.

The Bacillus of Malignant Œdema, or vibrion septique of Pasteur, occurs in man in a condition of inflammatory œdema associated with emphysema, and ultimately followed by gangrene, which is known as malignant œdema.

The bacillus, which is about $3\,\mu$ long and $1\,\mu$ broad, occurs in the form of rods or long filaments, some of which are motile. It forms spores, which are generally situated in the centre, sometimes towards the end, of the bacillus, and these are oval in shape, and somewhat thicker than the body of the bacillus. They do not stain by Gram's method. Spores never occur in the thread forms, only in the rod-shaped bacilli.

Comparing this bacillus with the anthrax bacillus, it

will be seen that the bacillus of malignant œdema is somewhat smaller in size; it has not the rigid cylindrical appearance; it possesses large oval spores, even in tissues, which cause the body of the bacillus to swell out in the centre, or towards the end; it possesses flagella, and is motile; it has no capsule, and does not stain with Gram's method.

The absence of a capsule is, according to Sobernheim, an important point in its differential diagnosis from the bacillus of anthrax.

The Spirillum of Relapsing Fever, or the Spirochæte Obermeieri.

This is a good example of a disease which can only be diagnosed with absolute certainty by means of the microscope.

Relapsing fever has an incubative period of about seven days, at the end of which an attack of fever occurs, lasting usually from five to seven days. This is abruptly terminated by a crisis, and is followed by an apyrexial period, or "first period of apyrexia," which usually persists for a week. At the end of this time a relapse, or second paroxysm, comes on, and is similar to, but is generally a day or two shorter, than the first attack. This in turn is followed by a "second period of apyrexia," which usually coincides with that of convalescence.

It is common to see two attacks of fever; much rarer to find one, or three, or four paroxysms.

The specific organisms appear in the blood shortly before an attack, rapidly increase in number during the pyrexia, and begin to disappear just before the crisis.

They may be found in very large numbers during the fever, but are entirely absent, or practically so, from the blood during the apyrexial period. They reappear at the next paroxysm, but disappear when that is over.

There is no relation between the number of spirochætes found in the blood and the intensity of the attack. (Wladimiroff.)*

The spirochætes are long, slender, spiral or sinuous filaments, measuring 10, 20, or 40 μ, or more in length, and at most 1 μ in breadth. The number of spiral turns varies from six to twenty. (Plate II. Fig. 10.)

"In fresh blood, the spirillum is seen to be flexible and very active. Its movements are progressive, with undulations passing wavelike along from one extremity to the other. It is so fine, that under a low power its presence is only revealed by the commotion amongst the blood corpuscles, which by the rapid movement of the spirilla are thrust violently aside." (Wesbrook).†

The ends of the spirillum are sharp and tapering.

Heydenrich distinguishes three movements in the spirilla, a twisting on the long axis, bending to one or other side, or a forward or backward movement of the whole organism.

Wladimiroff says that although many text books mention that the spirillum possesses flagella, this has not been proved.

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* Wladimiroff: "Rückfallfieber." Handbuch der path. Mikro-
organismen. Vol. iii., p. 78.

† Wesbrook: "Bacteriology of Relapsing Fever." Allbutt's System of
Medicine. Vol. i., p. 951.

The spirilla have a considerable degree of vitality outside the human body. Heydenrich found that their motility could be preserved for $2\frac{1}{2}$ to 14 days, according to the temperature at which they were kept, and in the bodies of those who had died during the crisis, they were found in a postmortem made 40 hours after death, although all their movement usually ceases 24 hours after the death of the patient.

In the blood of the living, spirilla may be found some hours before the crisis, and may persist through a pseudo-crisis, but after a true crisis they generally disappear rapidly, and are not to be found in the apyrexial period between two true attacks. (Wesbrook).

According to Carter, they may at their height be in the proportion of $^{1}/_{20}$ to $^{1}/_{10}$ the number of the red blood corpuscles.

Method of Examination.—The blood may be examined in the fresh state, when the movements of these characteristic organisms can be seen, or in dry stained film preparation.

To demonstrate them in the fresh condition, the lobe of the ear, or tip of the finger of a patient during the febrile period, is pricked, and the exuding drop of blood, caught on a clean cover-glass, held by forceps, is placed with the drop downwards on a slide, and the blood allowed to spread out by the weight of the cover-glass. Any movement of the red cells should attract attention, and with a suitable magnification, and with the iris diaphragm somewhat closed, these very transparent motile organisms may be seen moving between the corpuscles.

Sometimes, even with a high magnification, it is not easy to detect the spirilla, especially when the corpuscles are thick, and even in the clear spaces,

these fine threads have much the same refractive index as the medium in which they move, and can be overlooked. If it is desired to preserve the spirochæte, the blood should be collected in pipettes, and the end of these sealed in the flame.

Mamurowski places a drop of Müller's fluid on the finger, and pricks through this, so that the exuding blood mixes with the fluid, and is fixed at once. This is said to give very good results.

Weigert uses for the same purpose salt solution or osmic acid, and points out that the skin should not have been previously cleansed with alcohol, as this may cause coagulation of the blood, and so obscure the organisms.

Leptochinsky spreads thin films of blood, and examines these dry, without any further treatment, and in dry preparations the spirilla are said to be easily seen, but even here if the film be thick they may be obscured.

Albrechts treats the air-dried films several times with acetic acid, and then washes and dries them; by this means the hæmoglobin is removed, and only the nuclei of the leucocytes with their granules and the spirochæte are seen. The more active the movement of the spirilla before the film is made, the more cracked and irregular will they appear.

Staining.—Air-dried films, after fixation in alcohol and ether, may be easily stained with any of the ordinary aniline dyes, *e.g.*, thionine, methylene-blue, fuchsin, etc.

They may also be double-stained with eosine and methylene-blue, separately or combined, by Jenner's or Leishman's stain, as described under gonococci.

The films of blood may be either the ordinary thin

preparations, in which all the elements of the blood, as well as the parasite, are seen, or Ross's method of preparing thick films may be used, and either of these preparations can be very satisfactorily stained with Leishman's modification of Romanowsky's stain, as described in detail under plague bacilli (page 180).

Parasitic Fungi, affecting the Skin and Hair.

The following fungi are those with which we are chiefly concerned from a diagnostic point of view. In ringworm we find the small spored fungus, Microsporon Audouini, or the large spored fungus, Trichophyton megalosporon: in favus the Achorion Schoenleinii: in tinea versicolor the Microsporon furfur. Other rarer forms, *viz.*, the Microsporon minutissimum causing erythrasma, and Tinea imbricata, a tropical form of ringworm, also occur.

Microsporon Audouini, or Tricophyton Microsporon.

This, the small spored form, is by far the most frequent, causing 80 to 90 % of all forms of ringworm found in London. It attacks the scalp, face, and neck of children, not adults.

It is important to distinguish it from the other forms, as it is very refractory to treatment, and practically only attacks children.

With the naked eye it will be seen that almost every hair over an infected patch is diseased, fragile, or broken off, leaving comparatively long stumps.

The stumps have a white parasitic sheath extending over them throughout the intra-follicular portion, and for 3 mm. beyond the exit of the hair (Colcott Fox).*

Microscopically. This is a small fungus affecting both the inside and outside of the hair. The fungus on the outside consists of spores over the bulb, and extending round the shaft of the hair up to the broken end, like a bark.

Inside, the fungus forms segmented mycelia, which may be beaded. These mycelia attack chiefly the superficial parts of the hair, but terminate just above the bulb in a very characteristic fringe, and it is at this point that the hair is very apt to break. In the scrapings the fungus is seen as coarse and irregularly branching mycelia, which, although varying in length, are generally short. The branching usually takes place at right angles to the main thread.

According to Sabourand the spores of this variety measure about 2 to 3 *u*. Jamieson says that the mycelium is so fragile that it is seldom visible, but its special position is in the substance of the hair, and it forms an enveloping sheath round it. (Plate II. Fig. 13.) Spores, he remarks, are found both inside and outside the hair, in large masses.

Trichophyton Megalosporon, or large=spored Ringworm.

The spores in this form are larger, measuring 7 to 8 *μ* in diameter, and mycelium is always distinctly seen. It attacks the body, beard, nails, and sometimes the scalp, causing tinea barbæ and corporis, and also some forms of tinea capitis, in which position it is

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* Colcott Fox: *Brit. Med. Journal,* 1897. p. 876.

always much easier to cure than the small spored variety. (Plate II. Fig. 14).

It is easily distinguished from the T. microsporon by the large size of the spores and the presence of mycelium, but it may, on this account, be confused with the parasite of favus. (Jamieson).*

By some writers it is divided into the endothrix and ectothrix. Morris, however, thinks this is based merely on its accidental position, and may only depend on the degree of invasion. Both forms attack the root first and grow upwards, the mycelia are regularly jointed and branch dichotomously, and the spores are arranged in chains. Colcott Fox says that the endothrix form is distinguished with the naked eye, by the fact that there is no white sheath like the T. microsporon, and the stumps break off lower down, even in the mouth of the follicle, and become dark coloured and swollen.

The spores are placed round the hair, sometimes inside—sometimes outside, or both.

Malcolm Morris† states that whilst tinea circinata, ringworm on the body, has a characteristic appearance clinically, the discovery of the fungus is not always easy, even to an expert.

On the other hand, cases of ringworm of the scalp are sometimes difficult to diagnose by their clinical appearance, and can only be determined with certainty by a microscopic examination.

Method of Examination.—The affected hairs, which are broken, bent, or sometimes display a powdery sheath, are pulled out with a pair of forceps. It is of no use to pull out hairs indiscriminately.

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* Jamieson: Diseases of the Skin. Edin., 1894. 4th Edition, p. 554.
† Malcolm Morris: "Diseases of the Skin." London, 1903,

Duckworth and Behrend's chloroform test may help materially in the selection. If a few hairs from a case of ringworm of the head are placed on a slide, and a few drops of chloroform added, it will be seen, when the latter has evaporated, that all the hairs containing spores, or those in which the structure has been broken up by the parasite, assume a chalky whiteness. If chloroform be dropped on the head, after its evaporation, the skin of the scalp becomes white, and with a lens the diseased hairs are also seen to be white, whilst the normal hairs retain their usual colour. This reaction is more distinct in dark than in fair hair, and by its means the affected stumps may be seen and extracted. Jamieson* states that neither in chronic eczema, favus, or in any other condition is such an alteration produced by chloroform.

In ringworm of the beard, it is necessary to examine several stumps, and if crusts are present, suitable stumps may sometimes be found on their under surface.

In ringworm of the body, the margins of the rings should be somewhat deeply scraped with a scalpel, and the scales so obtained examined.

In ringworm of the nails, the affected part should be deeply scraped with a sharp knife, and the scrapings submitted to strong liquor potassæ for some time.

The hairs, stumps, or scales are placed on a slide, and if any ointment has been used on the affected part, treated with a little ether for a minute or so, and then with a few drops of filtered liquor potassæ. The slide should now be heated over a spirit lamp for half a minute, when the hairs, etc., may be

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* Jamieson : *loc. cit.*, page 560.

transferred to a watch glass of distilled water, and thence on to a slide, covered with a drop of glycerine, and a cover-glass put on the preparation. Sabourand uses a stronger solution of potash—*viz.*, 40 %, both for the hair and nails. In many cases I find it more convenient, especially when many scales are present, to remove the excess of potash after the preparation has been warmed, by means of filter paper, and then examine in glycerine, or even in the liquor potassæ itself.

This is all that need be done for diagnostic purposes, and the only possible fallacy is to mistake some of the small fat globules for spores.

For a detailed examination of the structure of the parasites, and for permanent preparations, the fungus should be stained.

MALCOLM MORRIS' METHOD, SLIGHTLY MODIFIED.

Hairs from the margin of a suspicious patch are extracted, and the root and shaft end about a quarter of an inch long, are cut off. These are washed in ether to remove the fatty matter, and are then stained with carbol - fuchsin, or carbol-gentian-violet, either on the slide, or in a watch-glass for about half - an - hour, during which the preparation should be heated for five minutes or so. Morris points out that the small spored fungus stains very quickly in about five minutes, whilst the large spored form takes much longer.

Pour off the stain, and add Gram's solution of iodine to the preparation, and let it remain five or ten minutes.

Dry the preparation with filter paper, and decolourise for a few seconds with aniline oil, or " with a mixture

of two to four drops of nitric acid in aniline for ten to fifteen minutes." It may, instead, be placed in aniline oil to which iodine has been added, until the solution has a deep mahogany colour.

It is then washed in xylol, and mounted in Canada balsam.

A simpler method of making a permanent stained film is, after making a diagnosis in liquor potassæ, to remove the cover-glass, and gently wash the preparation with water, or with 15 % alcohol in water, remove the fluid with filter paper, and then let the preparation dry in the air. When thoroughly dry, it may be treated as an ordinary film preparation, fixed by heat or alcohol, and stained by Gram's method, or its modification.

Achorion Schonleinii.

Favus is very rare in England, but until recently not uncommon in Scotland.

In order to prepare a specimen for microscopic examination, the hairs and crusts from a favus cup or scutulum, as it is called, should be treated in exactly the same way as was described for ringworm.

When teased out with liquor potassæ the favus cup is seen to consist of branching mycelia and spores, mixed with granular débris and pus corpuscles.

The spores are round or oval, measuring 3 to 8 μ long, and 3 to 4 μ broad, and may occur singly or in chains. The centre of a favus cup is mainly composed of spores, the margin of mycelial threads.

The mycelium, which is made up of shorter pieces than the tricophyton, forms a dense feltwork, in which spores are found. Its protoplasm is somewhat granular, and the breadth of the filament variable.

It will be noticed that the structure of the affected hair is not as much broken up by this parasite as in ringworm, and the mycelium runs for the most part in the long axis of the shaft. (Plate II., Fig. 15).

"Hairs, affected with favus, have none of the ragged outline, nor the dissected aspect that those in tinea tonsurans have, and consequently they, when they do break, do so at a much greater distance from the skin." (Jamieson).

The parasite of favus in the hair is distinguished from that of ringworm of the head, by the absence of the chalky whiteness when chloroform is applied, there is more mycelium and fewer spores, and the spores are more generally oval than round.

Microsporon furfur.

If the somewhat characteristic pale fawn or brown patches of pityriasis or tinea versicolor are scraped with a knife, and the scales so obtained placed on a slide, covered with a drop of liquor potassæ, and the cover-glass applied, this fungus will be readily recognised.

Should it be desirable to make permanent preparations, the following method, recommended by Joseph and Loewenbach* may be used. It is equally applicable for ringworm, and for the microsporon minutissimum of erythrasma.

The epithelial scrapings are subjected to the action of a mixture of alcohol and ether for twenty-four hours, then treated with glacial acetic acid for about five minutes, and placed on a slide, where the pieces

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* Joseph and Loewenbach: Technique histologique appliquée à la Dermatologie: Translated by Minne. Paris, 1902, p. 119.

are teased out with needles. The slide is then held over a flame, in order to slowly evaporate the acetic acid, and the preparation allowed to dry in the air. They may then be treated as dry film preparations, and stained with methylene-blue or by Gram's method.

Microscopically, favus is at once distinguished from a secondary syphilide, which it somewhat resembles, by the characteristic appearance of interlacing mycelium, inclosing groups of round spores. (Plate II. Fig. 16.) The mycelium consists of short thick curved hyphæ, measuring about 7 to 13 μ long, and 3 to 4 μ broad.

The spores, which are round, occur in clusters or groups, generally singly, seldom in chains, and measure 4 to 7 μ in diameter.

Plaut* says that in stained preparations, darkly coloured globules may be seen inside the spores, lying close to, but not actually in contact with the capsule, whilst the rest of the protoplasm is either colourless or faintly stained. Not infrequently, the spores are seen to have ruptured, setting free the deeply stained granules.

Microsporon minutissimum.

Erythrasma, a rare disease characterised by the presence of brown patches, especially in the genito-crural region, in the axilla, and between the nates, is associated with a parasite very like the M. furfur, except in the minute size of the spores and filaments. According to Jamieson, to demonstrate it properly, carefully stained preparations and a magnification of 700 diameters are necessary.

The mycelia, very fine twisted filaments which form

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* Plaut : " Die Hyphenpilze." Handbuch der path. Mikro-organismen. Vol. i., p. 652.

figures of S or V, are branched and distinctly septate, a single member of which measures, according to Sabourand, 5 to 15 μ long, and 0·8 to 1·3 μ broad. Among the branched filaments are found groups of exceedingly small, round, or somewhat angular spores.

It is distinguished from M. furfur by the small size of the elements, and both microsporon furfur and minutissimum are distinguished from all the other skin parasites by the fact that the spores occur in groups, and are never distributed uniformly throughout the mycelium.

Serum Diagnosis.

If the blood serum, in certain dilution, from a patient suffering from typhoid or Malta fever be added to an emulsion of living typhoid or Malta bacilli, these organisms will be seen under the microscope to be aggregated or collected into clumps, the agglutination reaction. If the experiment has been performed in suitable glass tubes, a sedimentation reaction occurs, and this is visible to the naked eye.

Widal's agglutination or sedimentation reaction may, therefore, be either a microscopic rapid or a macroscopic slow test.

The method is one of the greatest use for the practical diagnosis, particularly of typhoid and Malta fever.

Those who have at hand cultures, media, and an incubator, use fresh cultures of bacilli, but, as Widal pointed out, the diagnosis can be made with an emulsion of dead bacilli.

Collection of the Blood.—The simplest method of obtaining blood serum, is that suggested by Cabot.*

The most dependent part of the lobe of the ear is punctured with a triangular pointed needle, and the ear is then "milked strongly downwards towards the punctured spot," into a very small test-tube, which need not be sterilized. In this way, fifteen drops of blood are easily obtained, and will yield two or three large drops of serum. As soon as the blood has coagulated—a matter of two or three minutes—the clot is separated from the sides of the test-tube by means of a piece of wire, or blade of a penknife, and the serum then escapes and can be collected.

Blood may also be collected in capsules, as Wright† has suggested. These are made by heating a piece of glass tubing in two places, and drawing it out into capillary tubes, one end of which is bent up so as to form an acute angle with the capsule. The capsule is half filled with blood by capillarity and gravity. Both ends of the capsule are sealed by heating in the flame of a match or spirit lamp. When the serum has separated from the clot, it is aspirated into a capillary tube.

The blood may also be collected on the surface of a slide, piece of note paper or blotting paper, and afterwards dissolved in a drop of water.

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* Cabot : Serum Diagnosis : 1899.
† Wright : *Brit. Medical Journal*, Feb. 5th, 1898. p. 355.

The next step depends on whether we have at hand an incubator, media, and a reliable culture of the typhoid bacilli.

If so, a tube of neutral bouillon is inoculated with some of the latter, and incubated for not more than 24 hours, at a temperature of 37°C.

The bacilli should be actively motile and free from clumps, and in order to determine this, a drop of the bouillon culture should therefore be examined under the microscope. If clumps are present, some of the culture should be filtered through a filter paper, moistened with distilled water, as suggested by Hewlett.

Sheridan Delépine* says :—" When the typhoid bacillus is reinoculated in neutral bouillon daily for weeks or months, there comes a time when, all of a sudden, all the cultures will clump spontaneously on the addition of any serum."

Having thus obtained serum, and an evenly turbid bouillon culture, the next thing is the dilution of the serum.

Dilution of the Serum.—The question of the degree of dilution advisable, should always be considered with the necessary time limit, and Hewlett† states that "the dilution should not be less than 1 in 30, nor for convenience, more than 1 in 50, with a time limit not exceeding one hour."

Muir and Ritchie‖ say that if serum diluted 1 in 30 causes clumping in half-an-hour, it is certainly from a case of typhoid, and doubt should exist if a lower dilution or a longer time is necessary.

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* Sheridan Delépine: *Brit. Medical Journal*, 1897. Vol. i., p. 969.

† Hewlett: Bacteriology, 1902. 2nd edition, p. 278.

‖ Muir and Ritchie: Bacteriology. 3rd edition, p. 326.

The dilution may be made with neutral bouillon, normal serum, or most conveniently with physiological salt solution.

The easiest method of measuring the half dilution, *e.g.*, 1 in 15, is by means of a graduated capillary pipette, such as the white blood corpuscle pipette. It can also be done in ordinary capillary pipettes, as Wright has suggested. The serum is drawn up to a point marked on the stem of the pipette, and this is blown out into a watch glass. Equal quantities of normal salt solution are drawn up to the same mark, until the necessary dilution is obtained.

The dilution can also be made with a platinum loop, as Delépine suggests.

One drop of the diluted serum is then mixed on a slide with one drop of the bouillon culture, covered with a cover-glass, or a hanging drop preparation may be made, and examined under a magnification of about $^1/_6$ inch objective.

When examined thus, it will be seen that the bacilli are very soon motionless, in a short time they begin to collect into little heaps, and at the end of half-an-hour or so, very few free bacilli will be seen outside the clumps. Sometimes a bacillus at the margin of a clump will appear spinning round one of its ends.

When the reaction is feeble, or just about to begin, the bacilli are drawn into very loose groups and do not actually touch one another, and the movement of the other bacilli may not actually cease, but become sluggish. At the end of the time limit—half to one hour—the clumping will be quite distinct if the case be one of typhoid fever.

Instead of living typhoid bacilli, an emulsion made

from dead cultures can be used in exactly the same way as just described, but of course no movement is present, and one must look for clumping of the dead bacilli.

Dead cultures are usually employed in the sedimentation methods.

In addition to this method, which is usually spoken of as the microscopic or rapid one, a macroscopic or sedimentation method may be used with living or killed cultures of these bacilli. Wright and Semple* have described in detail the method of serum diagnosis, by dead cultures, of typhoid and Malta fever. As they say, the diagnosis can by this means be made by any practitioner at home or abroad, without the need of an incubator, cultures, or even a microscope. " He will be able to carry about these cultures without risk, and he will not need to take precautions against infection when he is employing them in the serum diagnosis of doubtful cases of fever. His whole equipment in connection with serum diagnosis may, in fact, be narrowed down to a supply of these dead bacteria, a small supply of glass tubing of, say, $^{3}/_{16}$ to $^{1}/_{4}$ inch diameter, and the blowpipe apparatus which is described."

At my request the Director of the Lister Institute of Preventive Medicine, Chelsea Gardens, S.W., has kindly promised to supply, at a very small cost, dead cultures of these bacteria ; and sero-sedimentation tubes and capsules for collecting the blood (full directions for making these will be found in *The Lancet*, Vol. I., 1903, and the *Brit. Med. Journal*, Vol. I., Feb. 5, 1898) may, if it is not convenient to make them at

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* Wright and Semple : *Brit. Med. Journal*, 1897. Vol. I., p. 1214.

home, be obtained from A. E. Dean, Jun., 73, Hatton Gardens, E.C.

The following is, briefly, the method adopted in using sedimentation tubes. One of the tubes is marked on the stem with a red wax pencil, or ink, and the undiluted serum drawn up to this mark. This is then blown out into a watch glass, and normal saline solution is drawn up to the same point four times, and this blown out and mixed with the serum, so that we have now a dilution of 1 in 5. With this 1 in 5 dilution, we can, by similar means, make a 1 in 15 diluted serum, and equal quantities of this and the dead culture fluid are drawn into the capillary sedimentation tube, mixed in the bulb of the same, and the mixture again driven down into the capillary stem, The orifice of the latter is then sealed in the flame, and the tube placed in a vertical position for twelve hours. A control tube of equal volumes of the bacterial culture and normal saline solution should be made at the same time.

When examined at the end of the specified time, the fluid in the control tube has remained turbid throughout. In the tube containing the typhoid serum, the upper layer of the fluid has become clear, and a flocculent precipitate has fallen to the bottom of the tube. "A less complete positive reaction would have manifested itself in the formation of a precisely similar flocculent precipitate in an incompletely clarified fluid."

It is advisable in using the sedimentation tubes to use serum without any corpuscles, and normal saline solution, and not distilled water, as these may cause a little confusion.

Prof. Wright* has recently said that "if we accept

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* A. E. Wright: *Lancet*, Vol. II., 1903. July 25.

as adequate an agglutination altogether inconspicuous to the naked eye, and if we allow a considerable interval for its appearance, we must exact a high degree of serum dilution. But reflection will make it clear that we can maintain exactly the same strictness in our standard, if we relax in the matter of our dilutions, and become correspondingly more exacting in other respects. Our exactions may, for instance, take the form of demanding that the agglutination effect shall manifest itself instantaneously, that the agglutination effect shall make its appearance in dense bacterial suspension, and that it shall be so pronounced as to discover itself to the naked eye." By this means, he says, " we dispense with all necessity for a microscope, and we obtain our results with an absolute minimum of delay—in the case of an ordinary typhoid serum, practically instantaneously. I may note that such an instantaneous and macroscopically visible agglutination, obtained in a mixture of equal parts of undiluted serum and typhoid culture, is, in my experience, quite as conclusive of the diagnosis as an agglutination visible under the microscope after a quarter-of-an-hour in a 1 in 50 dilution of the serum."

In a private communication, Prof. Wright, speaking of the dilution advisable for use with the dead typhoid cultures, says :—" I think dilution of the serum for purposes of diagnosis of typhoid is unnecessary."

The serum diagnosis of Malta fever may be carried out with dead cultures in the same way as the above.

At present, the serum diagnosis of tubercle by an emulsion of tubercle bacilli cannot be considered as a practical diagnostic method. On this subject, Wright*

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* Wright: "A note on the Serum Reaction of Tubercle." *Lancet*, 1903. Vol. i., May 9.

says :—" Bearing in mind, in particular, the fact that tuberculous infections are for the most part purely localised infections, we have little reason to expect to derive much assistance in the diagnosis of tubercle from the indications of the serum sedimentation reaction. In point of fact, in contrast with what is the case in septicæmic diseases, such as typhoid and Malta fever, very little diagnostic importance attaches to the negative result of a serum sedimentation test in the case of tubercle. Important, on the other hand, in connection with the serum sedimentation reaction, is the fact that it gives, in the case where the patient is being subjected to therapeutic inoculations of tubercle vaccine, some indication of the extent to which anti-bacterial substances are being elaborated in the organism."

In the diagnosis of typhoid it must be remembered that occasionally, though very rarely, the agglutination reaction may be absent throughout the whole course of a definite attack of enteric fever.

Generally speaking, the reaction is not obtained earlier than the sixth or seventh day of the fever, and sometimes considerably later than this. The serum of a patient who has had an attack of typhoid, or who has been inoculated with Wright's serum, may show the reaction for a very considerable time after, and it is of importance to remember this fact in making a diagnosis of a doubtful case.

If the blood fails to give a reaction when tested on three occasions, at intervals of a week, it is improbable that the case is one of typhoid. (Hewlett.)

Certain strains of typhoid bacilli, and certain conditions of the culture media, may give somewhat variable results. The serum in some cases of illness due to

the para‑typhoid or para‑colon bacilli, which really belong to the bacillus enteritidis group of organisms, may occasionally give an agglutination reaction, and even normal serum will generally agglutinate in a dilution of 1 in 3 or 4, and sometimes in greater dilutions. (Hewlett.)

Notwithstanding these facts, the serum diagnosis of typhoid fever is of extreme value, and now that dead cultures of typhoid bacilli can be so easily obtained, and the method so simplified, it is within the reach of all practitioners.

FICKER'S METHOD* OF SERUM DIAGNOSIS OF TYPHOID.

Ficker, of the Hygienic Institute of the University, Berlin, realising the importance of the Gruber-Widal serum test of typhoid fever, has simplified the process, and adapted it to the wants of the practitioner.

In order to render the method as practicable as possible, he has kept the following points in mind :—

"1. The use of living typhoid cultures for the reaction must be dispensed with, as the physician does not possess the necessary outfit for work with infectious cultures.

"2. The preparation containing the specific agglutinating body must be stable, and free from living typhoid bacilli.

"3. It must not tend to clear spontaneously during the time necessary for the reaction.

* M. Ficker: Ueber ein Typhusdiagnosticum. *Berliner Klin Wochenshr.* 1903. No. 45, Nov. 9, p. 1021.

"4. The reaction must be quite evident to the naked eye, and absolutely definit e in its result.

"5. The reaction must not require continued observation for two hours, as the usual laboratory method does, as this loss of time is not possible in practice.

"6. It must be carried out at ordinary room temperature, without the necessity of an incubator.

"7. The preparation must be influenced by the measured dilutions of serum, in precisely the same way, and in the same degree as is the living suspension of the typhoid bacillus."

Ficker states that he has succeeded in preparing a fluid which is capable of replacing the culture of living typhoid bacilli. The third and seventh conditions were very difficult to fulfil, but he has managed to overcome these. His diagnostic preparation is a slightly turbid sterile fluid, which can be kept for more than nine months, if preserved in a dark and cool place. It ought to be shaken up from time to time and especially before use.

He advises that the serum which is to be examined should be diluted with ten times its volume of sterile physiological salt solution, and 0·2 cc.m. and 0·1 cc.m. of the diluted serum, measured with a graduated pipette, is placed in pointed glass tubes, labelled No. 1 and No. 2. The tube No. 1 contains 0·8 cc.m. of the diagnostic fluid, and No. 2 tube, 0·9 cc.m. A third tube contains 1 cc.m. of the diagnostic fluid, without the addition of any serum. The tubes are then corked up, and the contents well shaken. They are

then set aside at ordinary room temperature, but excluded from light. After ten, twelve, or fourteen hours, the results will be clear ; after twenty hours, a certain result ought not to be expected.

The positive reaction, seen by holding the tubes against a dark background, or in front of the light, consists in a distinct clearing up of the turbidity of the fluid, while if pointed glass tubes have been used, the clumping together of the agglutinating substance into balls will also be seen.

Ficker claims that his preparation enables the practitioner to carry out the agglutination test for enteric fever without living cultures, without an incubator, and without a microscope, and says that the results which have been obtained in some large clinics testify that the method is reliable.

The diagnostic fluid and complete outfit can be obtained from E. Merck, 16, Jewry Street, E.C.

J. Meyer[*] has compared the results obtained by Ficker's preparation with that of Widal's method, in which living cultures are used. He finds that both methods gave exactly the same results, and control tests on non-typhoid blood were equally satisfactory. He thinks that Ficker's method is simple to carry out, and is not apt to give discordant results owing to differences in the activity of the cultures used, as is the case in the Widal reaction, done with living typhoid bacilli.

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[*] Meyer : *Berliner klin Wochenshr.* 1904, Feb. 15,

Cytodiagnosis.

Cytodiagnosis, a term applied to the determination of the nature and variety of cellular elements found in an exudation, has been elaborated largely by Widal and Ravaut, in France, and Wolff, in Germany; and numerous communications have appeared on the subject since 1900. It has already proved to be a valuable additional aid to diagnosis in England, and although it does not properly belong to the domain of bacteriology, yet it is intimately associated with the bacteriological examinations of exudations in general.

In the fluid, drawn off from a case of pleurisy with effusion, it is well known that the search, for example, for the tubercle bacillus, is very difficult and usually unsuccessful, and yet very valuable assistance may sometimes be obtained by an examination of the cellular elements which may be present in it, and the results so obtained may materially assist in the diagnosis, prognosis, and treatment of the case.

In addition to pleural effusions, the cerebro-spinal, pericardial, peritoneal, and arthritic fluids can be examined.

The fluid to be examined may be that drawn off by an aspirator, or an aseptic syringe.

Any of the ordinary hypodermic or diagnostic syringes in common use answer very well, but they should be provided with a sharp needle of about two to three inches long, preferably made of platinum iridium, as this can be sterilized in the flame without injury.

If a bacteriological examination is also to be made of the fluid, the whole syringe should be sterilized, but otherwise it will be sufficient to sterilize the needle only. Of course the skin of the part should be washed with soap and water, then with some antiseptic, as is ordinarily done.

The position at which the puncture is made, depends on the nature of the case, and need not be further specified here.

If the fluid so obtained is purulent, a few films of the pus should be spread in the usual way, and may then be fixed and stained. In pus, especial attention should also be paid to any bacteria present.

If the fluid is *serous*, and is seen to contain a fair number of cellular elements, it will not be necessary to centrifugalise it. It should be poured into a conical glass (that recommended for obtaining urinary deposits, described under tubercle bacilli in urine, being particularly convenient), and films made from the sediment.

If the fluid be apparently very free from cells, it may either be placed in the conical glass (Fig. 3, page 103), especially if there is a large quantity of it, *e.g.*, from a pleurisy with effusion, or it may be necessary to centrifugalise it.

In order to prevent coagulation, Widal and others defibrinate it before placing it in the centrifuge. The fluid, immediately after it is withdrawn from the body, is placed in a bottle or strong flask, containing a number of glass beads, and thoroughly shaken until a clot is formed, which takes place after about fifteen minutes to an hour. If the exudation fluid has already undergone coagulation, it is poured with the clot into the flask containing the glass beads, and shaken up for about twelve minutes as before.

Wolff* says that the fluid should be examined as soon as possible after it has been withdrawn. If this is impossible, coagulation may be prevented by the addition of citrate of soda. He does not recommend Widal's method.

The sediment obtained in either of these ways is spread out on slides in the usual method of preparing films, allowed to thoroughly dry in the air, and they can then be fixed.

The method of fixing and staining is practically the same as that for blood films generally.

The air-dried films can be conveniently fixed by placing them in alcohol and ether for about one to ten minutes, and then stain them with eosine and methylene separately, or together with Jenner's stain. In the latter case, it is essential that the films should not have been previously fixed, but merely dried in the air. Unfixed air-dried films may also be stained with Leishman's modification of Romanowsky's method. These three methods have been described under the gonococci and plague bacilli.

By far the simplest plan for those who are not accustomed to these aniline dyes is to stain the films with eosine and hæmatoxylin in the following way :—Films, fixed by immersion in equal parts of alcohol and ether for a few minutes, are placed in a dilute water-alcoholic solution of eosine—*viz.*, eosine, 1 part; water, 100 parts; alcohol, 100 parts — for about a minute. They are then washed in water and dried.

Hæmatoxylin, Böhmer's or Delafield's solution, which can be bought ready made, is then filtered on to the slide, and allowed to remain ten to sixty seconds,

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* Wolff: "Exudate and Transsudate." Encyklopädie der Mikroskopischen Technik. Berlin, 1903. p. 302.

according to the staining power of the solution. The film is then washed in water, and examined whilst wet, to see if the blue of the hæmatoxylin in the nuclei of the leucocytes is sufficiently deep; if not, they are again exposed to the hæmatoxylin.

The films are then well washed, and even allowed to soak, in tap water, which is sufficiently alkaline to develope the blue of the hæmatoxylin, dried, and mounted in oil or balsam.

I usually stain three such films — one with eosine and hæmatoxylin, one with Jenner's, and one with Leishman's stain.

The elements found in films of the sediment will be the leucocytes of the blood, especially the polymorphonuclear and lymphocytes of varying size, probably also some red blood corpuscles and endothelial cells. The leucocytes found will, in fresh effusions, exactly resemble those of normal blood, but in older exudations they will probably show considerable alteration, the protoplasm having undergone disintegration or degeneration, and the nuclei have often lost their intranuclear network, and stain indifferently, or sometimes deeply, with the basic dyes; but, as a rule, it will not be difficult to classify them, provided that the pus or fluid has not been kept too long before it is examined.

In the following account of the changes found in the exudations, I have made free use of a small work by Labbé: "Le Cytodiagnostic."

Normal serous fluid, taken from a serous cavity, contains white and red blood corpuscles, the former being considerably in excess of those found in the blood. The white cells consist of multinucleated or

polymorphonuclear leucocytes — *i.e.*, cells with neutrophile granules and many lobed nuclei; uninucleated leucocytes or lymphocytes of varying size, containing one relatively large nucleus and no granules; and eosinophile leucocytes, containing large granules in their protoplasm, which stain deeply with the acid aniline dyes such as eosine. A detailed description of these elements has been given elsewhere.*

In addition to the leucocytes, a few endothelial cells will generally be seen.

Serous fluids from the pleural or pericardial cavity have much the same characters, whilst those from a joint generally contain fewer cells.

(A) **Pleural Effusions.**

1. *Primary Tuberculosis of the Pleura* is at its height generally associated with a great increase in the number of the lymphocytes, mixed with a considerable number of red blood corpuscles. (Widal, Litten, and Wolff).

During the first few days of the effusion, large uninucleated cells which have been regarded as desquamated epithelial cells, or as large uninucleated leucocytes, may be found in small numbers, but these disappear in a few days. Multinucleated and eosinophile cells may also be present during this early stage, but they never, according to Widal and Ravaut, exceed 10 % of the elements present.

2. *Secondary Tuberculosis of the Pleura*, associated with pulmonary tuberculosis, is characterised by the small number of cells present, and by the alteration of these. They have undergone fatty degeneration

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* Coles : The Blood." Churchill : 2nd edition, 1902.

and appear irregular, filled with vacuoles, and contain granules which stain black with osmic acid.

Red blood corpuscles are present in very small numbers, the lymphocytes are deformed, and the multinucleated cells are so altered that, according to Labbé, they are only recognisable by their neutrophile granules.

"The nucleus of the multinucleated cells have undergone a degenerative change, which is spoken of as 'pycnose.' It is broken up into several ball-like masses, which stain deeply and uniformly, and appear completely separated from one another. Endothelial cells united in masses are never met with. Generally in these cases, the background of the preparation is deeply stained, and it is very difficult to distinguish the outline of the leucocytes, the protoplasm of which stains the same colour as the background." (Labbé).*

According to some authorities, secondary pleurisies tending towards recovery, become lymphocytic, but when they run into a chronic condition, as is usually the case, the exudation only contains degenerated and altered elements.

Generally speaking, tubercular exudations are very constant in their cellular nature, but sometimes in the secondary tubercular pleurisies there may be a marked increase in the percentage of the eosinophile cells.

3. *Aseptic Pleurisy in Cardiac and Renal Disease.*— These are essentially characterised by the presence of endothelial cells. These are usually found in groups of eight to ten elements, so fused together that the margins of the cells are usually obliterated, and the individual cells only recognised by their nuclei, which

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* Labbé: Le Cytodiagnostic. Paris, 1903, p. 46.

are generally not very distinctly stained. Sometimes the epithelial cells occur singly or in pairs, and in cases of recent hydrothorax the endothelial cells are occasionally so abundant as to cover the whole field of the microscope.

Later on lymphocytes may appear, and the fused masses of endothelial cells diminish somewhat in number, become dropsical, hyaline, and disintegrate.

In congestion or infarcts of the lung occurring in the course of heart disease, red blood corpuscles and multinucleated leucocytes are added to the endothelial and lymphocytic cells.

4. *Cancerous Pleurisy.* — The cellular elements here are inconstant. Sometimes malignant cells may be found in the fluid. These are larger than the endothelial cells with which they are often associated, and frequently contain granules and vacuoles. Their nuclei, which are very large and oval, often show two or three very large and distinct nucleoli.

In other cases, merely groups of endothelial cells, lymphocytes, multinucleated cells, and generally a large number of red blood corpuscles are seen.

In a case of pleurisy occurring during secondary syphilis, Widal & Ravaut found 35 % of endothelial cells, 22 % lymphocytes, 37 % large uninucleated eosinophile cells, and 6 % of large uninucleated cells with neutrophile granules. It may be noticed that the last two elements do not occur in the blood in health, only in the marrow. They are characteristic of leucocythæmia when found in the blood.

5. *Septic Exudations. Pleurisy due to Pneumococci.* —These are characterised by the presence of multi-nucleated leucocytes, which are present in numbers directly proportional to the number of pneumococci.

These leucocytes, often associated with the presence of endothelial cells, may swell up and appear very like a large uninucleated leucocyte.

When such a pleurisy tends towards recovery, the multinucleated elements disappear, and some lymphocytes present themselves. When, on the other hand, it goes on to suppuration, the multinucleated leucocytes become very abundant, their nuclei divide up into balls (pycnosis), and they might be mistaken for lymphocytes, except for the presence of neutrophile granules in the protoplasm.

Pleurisy due to Streptococci is associated with a large number of multinucleated and isolated endothelial cells.

It will thus be seen that the general rule is that tubercular disease is associated with lymphocytes, pyogenic diseases with multinucleated leucocytes, and the reaction to mechanical irritation with endothelial cells.

(B) Peritoneal Fluid.

The cellular elements found here are not nearly as constant as in the pleural exudation. In ascites due to mechanical causes, endothelial cells and a few leucocytes are found.

In malignant disease of abdominal organs, occasionally cancer cells may be met with in the peritoneal fluid, but more frequently endothelial cells are found.

In tubercular peritonitis, sometimes only lymphocytes are seen, but Widal and Ravaut have generally found multinucleated cells.

(C) Cerebro=spinal Fluid.

Lumbar puncture, as devised by Quincke, was used for therapeutic as well as diagnostic purposes. It is,

with ordinary care, quite a safe little operation, but it must be remembered that it is, from all accounts, somewhat dangerous to withdraw a large amount of fluid—*i.e*, over an ounce, in cases of cerebellar tumour. I would refer the reader, for details of the method of its performance, either to Labbé's book already mentioned, or to two articles on cytodiagnosis in nerve disease by Dana and Hastings, and also Fraenkel, in the same number of the *Medical Record* (New York, Jan. 23, 1904, page 121, *et seq.*)

The fluid which comes through the needle need not be defibrinated, but, as it contains very few elements, it should be immediately centrifugalised. Films are prepared from the sediment, and stained exactly as previously described.

The cerebro-spinal fluid in health, or even in acute diseases which are unattended with meningitis, contains no cells, or, at most, a few red blood corpuscles and leucocytes.

In *tubercular meningitis* the cerebro-spinal fluid contains a varying number of cells, which are usually nearly all lymphocytes, with a very few multinucleated leucocytes and red blood corpuscles. In acute meningitis, due to the pneumococcus, meningococcus, or streptococcus, the fluid contains only multinucleated leucocytes mixed with lymphocytes and red blood corpuscles.

According to Labbé, in fatal cases the multi-nucleated leucocytes persist till death, whilst in a curable case these cells persist during the acute stage, and are gradually replaced by uninucleated cells, and later on, when recovery has taken place, the fluid has returned to its normal appearance.

Each relapse is attended with an increase in the

multinucleated leucocytes. From this it will be seen that if cerebro-spinal fluid was taken from a case somewhat late in its course, and lymphocytes found, this might be thought to indicate a tubercular condition. To determine whether the case was one of tubercular or recovery from an acute meningitis, one must be guided by the clinical features of the case, and also by an examination of stained films made from the sediment for the specific organism, remembering, however, that the tubercle bacillus is not readily found in this fluid.

In tabes, and in general paralysis, the cerebro-spinal fluid contains a large number of cells, especially lymphocytes, some large uninucleated leucocytes and endothelial cells. Generally speaking, the occurrence of lymphocytosis is fairly constant.

As a general rule Labbé states that in organic diseases of the nervous system in which the meninges are in a state of inflammation, when the process is acute, a multinucleated leucocytosis is the rule, and this is followed, on its recovery, by, at first, an uninucleated leucocytosis, and finally, a normal condition.

When the process is chronic, lymphocytosis is the rule, e.g., in syphilis. Peripheral neuritis, or cerebral tumours, which do not touch the cortical portion of the brain, cause no meningeal irritation, and therefore lymphocytosis is absent.

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