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THE EFFECT OF TUBERCULIN TREATMENT UPON
THE MORPHOLOGY OF THE TUBERCLE BACILLUS
IN THE SPUTUM.

Thesis for the degree of M.D.

by

Arthur Murray Masters,

M.B., Ch.B., Edin. 1910.

1st March, 1914.



Objects of the Thesis.

The main object of the investigations on which this Thesis was based, was to determine whether or not therapeutic injections of tuberculin had any influence on the microscopic characters of the tubercle bacilli found in the sputum of tuberculous persons.

Two staining methods having been employed for the bacilli (the Ziehl-Neelsen stain and Much's modification of Gram's stain), comparisons were made to determine whether any more bacilli were stained by one method than by the other.

My thanks are due to Sir Robert Philip for suggesting the line of work I have followed up, for permitting me to work in the laboratory of the Royal Victoria Dispensary for Consumption, and for the facilities afforded me for obtaining specimens of sputum.

I also desire to thank Dr. James Miller for supervising the investigations, and for his advice as to the method in which the work should be carried out.

Characters of the Tubercle Bacillus.

Tubercle bacilli appear in the form of slender rods from 2 to 4μ in length, and $.3\mu$ in width. Occasionally forms are met with up to 15μ or more in length, both in cultures and in the tissues. They may be straight or slightly curved, and their ends are usually rounded. Their diameters may be uniform throughout, or they may appear beaded or irregularly stained.

Two bacilli are often found attached end to end, and usually form in such cases an obtuse angle.

Metchnikoff, Coppen Jones and others have shown that sometimes in the secretions, and always in old agar cultures, certain of the bacilli become much elongated and filamentous, and also show true branching. Further, that in some cases the terminal ends of these forms present definite club formation. Evidence of these being degenerative forms is not considered sufficient.

These appearances suggested to Metchnikoff that "the bacillus as ordinarily met with is not an end stage, but only a stage in the developmental cycle of a filamentous fungus".

A number of bacteriologists have placed it with the streptothriciæ or actinomycetes, or as closely allied to these, and they prefer to speak of it as a "tubercle/

"tubercle fungus". More recent researches have only led to a more general acceptance of these ideas which have been placed on a sounder basis by the work of Babes and Levaditi, Friedrich, Schultze, Lubarsch, Moeller, Ledoux-Lebard and others.

Bruns expressed the view that the aberrant forms really belonged to the saprophytic vegetation of an organism which as a parasite appeared in the form of rods.

TYPES. It is now generally agreed that there are two chief types of the tubercle bacillus which differ both in their cultural characters and in their virulence - a bovine and a human type. The bovine bacilli, when cultivated, are shorter, thicker and more regular in size; whilst their growth on various culture media is scantier than that of the human type.

Although the majority of bacilli found in human tuberculosis are of the human type, bacilli are frequently found which, when cultivated, are indistinguishable by any known means from those obtained from bovine tuberculosis. Intermediate varieties are also met with. The type characters of the bacilli are not always constant.

Staining/

Staining properties.

Ordinary stains are taken up slowly and faintly by the tubercle bacillus, and to ensure satisfactory staining one needs to employ powerful solutions such as gentian-violet or Fuchsin, along with a solution of carbolic acid or aniline oil water.

The staining with these solutions requires to be carried out for a long time, and heat must be applied to accelerate the process. Tubercle bacilli share with some other organism the property of being "acid-fast", that is after staining has taken place they resist decolorisation by solutions which readily remove the colour from the tissues and from other organisms which may be present.

Ehrlich has suggested that the acid fastness is probably due to a substance surrounding the bacillus, which is permeable to aniline oil water, alkalis, etc., and impermeable to acids. The method of staining most commonly employed is known as the Ziehl-Neelsen method.

The procedure is as follows:-

1. Stain for 3 to 5 minutes with carbol-fuchsin.

Basic fuchsin	1 part
Absolute alcohol	10 parts
Solution of Carbolic acid (1 in 20)	100 parts.

Heat until steam rises.

2. Wash in water.

3./

3. Differentiate with sulphuric acid (20 per cent solution).
4. Wash in water.
5. Counterstain with saturated aqueous solution of methylene blue for half a minute.
6. Wash in water, dry and mount.

It has long been recognised that the older methods of staining might not always reveal the presence of bacilli in tubercular lesions.

Important observations by Much have been brought forward dealing with this subject.

He concludes that the tubercle virus exists in three forms:- (a) an ordinary bacillary form stainable by the Ziehl method; (b) a fine bacillary form which is not acid-fast, often showing granules in its interior; and (c) free granules which also fail to stain with the Ziehl method.

The two last forms can be stained by Gram's method, when the stain is applied for a long time, or when heat is employed. Much has employed stains with which he claims that bacilli and granules can be found in tubercular lesions when the Ziehl method gives a negative result.

Further, he found that when the bacilli from a culture were added to sterilised milk and incubated, acid-fast/

acid-fast forms disappeared, whilst those stainable by Gram's method remained; and that when this had occurred the milk, when injected into an animal, produced tuberculosis in which acid-fast bacilli were demonstrable.

Much gives three modifications of Gram's method, the following being the one which was used for these observations:-

1. Stain for five minutes with carbol gentian violet, heating until steam rises.

Gentian violet (saturated solution in absolute alcohol)	10 c.c.
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Carbolic acid (1 in 40)	100 c.c.
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2. Wash in water.
3. Gram's iodine 1 minute.
4. Hydrochloric acid (5 per cent) 1 to 3 minutes.
5. Nitric acid (3 per cent) 1 to 3 minutes.
6. Differentiate in alcohol and acetone in equal part for half a minute.
7. Wash in water.
8. Counterstain with Saffranin (1 per cent aqueous solution).
9. Wash, dry and mount.

Carl Spengler has described what he calls "splitter"/

"splitter" forms of the tubercle organism in sputa in which there were usually no normal bacilli to be found.

He concludes from his observations that these forms, which are acid-fast, are involution forms of the tubercle bacillus, somewhat devitalised and of a lower virulence; also that on suitable culture media they develop into bacilli.

When stained, the bacilli either take the dye uniformly or present a beaded appearance, portions of the bacilli taking the colour well, while the intervening parts remain unstained.

There has been a good deal of controversy concerning the nature of this want of uniformity in staining. The unstained portions were formerly thought to be spores, but they are now generally considered to be vacuoles. It is held that it is a sign of involution or degeneration when the bacilli do not stain uniformly, and it has been shown in support of this view that it is the older forms which show this inequality of staining.

The deeply-stained portions of the bacilli were also formerly considered to be spores. They do not however resemble spores as seen in other organisms. All known spores resist heat more strongly than the fully/

fully developed bacilli, but experiments have shown that these bodies are no more capable of resisting heat than the bacilli themselves.

In regard to the beaded appearances presented by the stained bacilli, in making comparisons between the Ziehl-Neelsen, Much-Gram and other methods of staining, some consider that it is always the same parts of the bacilli that show affinity for the different stains, while others hold that it is not always the same parts that stain more deeply.

Selection of Sputum Specimens.

The sputum examined for the purpose of these investigations was obtained from patients at the Royal Victoria Hospital for Consumption, Edinburgh, and also from patients receiving domiciliary treatment at the Royal Victoria Dispensary.

The sputum of twenty-eight patients was examined periodically, in most cases over a period of six months.

Some of these patients had not received tuberculin injections as part of their treatment. In the case of those who had tuberculin treatment, the sputum was first examined before it was begun, and afterwards at intervals while this treatment was being carried out/

out. Tuberculin Béranecq was the preparation employed in these cases. Sputum of patients who had already received tuberculin treatment over a considerable period, and who were still receiving injections was also examined periodically.

The sputum was not subjected to any concentration method before making the films.

Two films were prepared from each specimen of sputum, one being stained by the Ziehl-Neelsen method, and the other by Much's modification of Gram's stain.

Method of drawing the bacilli.

In order to facilitate comparisons between the specimens, drawings of the bacilli were made. For this purpose the Abbe' drawing apparatus was employed, and in order that all the drawings should be approximately on the same scale, the drawing paper was always kept at the same distance from, and on the same plane as the reflecting mirror of the drawing apparatus.

A scale was copied at the foot of each drawing in order that the length of the bacilli might be more readily compared.

For/

For this purpose a micrometer eyepiece was employed, and its scale copied by means of the drawing apparatus.

Each drawing represents specimens of the bacilli on one slide, an endeavour being made not to select any particular organisms but to draw any that were seen as the slide was moved, until a number sufficient for purposes of comparison was obtained. With the drawing camera in position each bacillus was faintly copied in pencil, the details being immediately copied in ink without the aid of the camera. The bacilli were coloured either red or purple to represent respectively the two staining methods employed - the Ziehl-Neelsen and Much-Gram methods.

Comparisons between the two staining methods.

In order to make comparisons between the above two staining methods with a view to determine more or less roughly whether more tubercle bacilli were stained by one than by the other, the following procedure was adopted. Two films prepared from the same sputum, and stained by the two methods were compared in each case. A square was drawn on the lens of the eyepiece of the microscope, and the bacilli/

bacilli seen within this square were counted.

Having selected portions of the films of about equal thickness, the bacilli within six fields in each slide were counted, and the number compared.

C O N C L U S I O N S .

1. That there is no apparent alteration in the size of tubercle bacilli in the sputum of patients who have had tuberculin treatment unless it is a slight diminution in size.
2. That tuberculin injections apparently produce no other alteration in the morphological characters, staining properties, etc., of the tubercle bacillus.
3. That in specimens stained by the Much-Gram method objects were frequently seen which somewhat resembled tubercle bacilli, but which could not be considered as such with any certainty.
4. That about 15 per cent more undoubted tubercle bacilli were stained by the Much-Gram than by the Ziehl-Neelsen method.
5. That in the case of the majority of patients who received tuberculin injections, the sputum became scanty more quickly, and the bacilli were found in fewer numbers after the injections had been continued for some time, than was the case in those/

those who received no tuberculin.

6. That in several cases it was noted that tubercle bacilli became more abundant shortly after the tuberculin injections were begun, and subsequently diminished in number more or less quickly.
 7. That the beaded appearance is always more marked when the bacilli are stained by the Much-Gram than by the Ziehl-Neelsen method.
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The following series of drawings represent tubercle bacilli from some of the patients whose sputum was periodically examined.

Examples are given from those patients who have received tuberculin injections since the sputum was first examined, those who have had no tuberculin being examined as controls, and one who had tuberculin over a considerable period before these observations were begun.

The drawings placed side by side were made from films from the same specimen of sputum, and stained by the two methods as described before.

G.P. male aet 32 years.

Domiliary treatment at the Royal Victoria Dispensary was begun on 4. vi. 1913.

There were well-marked bilateral pulmonary lesions, & moderate signs of systemic vitonication.

Tuberculin injections (TBK D₆ sec) begun on 28. vi. 1913 & continued weekly in increasing doses.

The patient's pulmonary & general conditions have markedly improved, & the bacilli have been much reduced in numbers in the sputum. The sputum has been examined twelve times during five months.



Ziehl-Neelsen.



Mach-Graws.

1st specimen 24. vi. 1913.

Tuberculin injections not yet begun.



2nd Specimen

9. VII. 1913

Tuberculin
injections
begun on
28. VI. 1913

20 5 10



20 5 10

Ziehl-Neelsen.

Muck-Graw.



3rd Specimen

17. VII. 1913

20 5 10



20 5 10

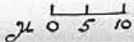
Ziehl-Neelsen.

Muck-Graw.

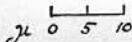


4th specimen

27. VII. 1913.



Ziehl-Neelsen

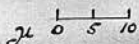


Much-Gram.

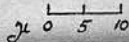


5th specimen

11. VIII. 1913.



Ziehl-Neelsen.

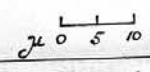
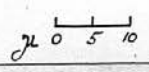


Much-Gram.



6th Specimen

26. VIII. 1913



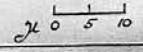
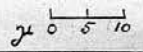
Ziehl-Nielsen

Much-Grav



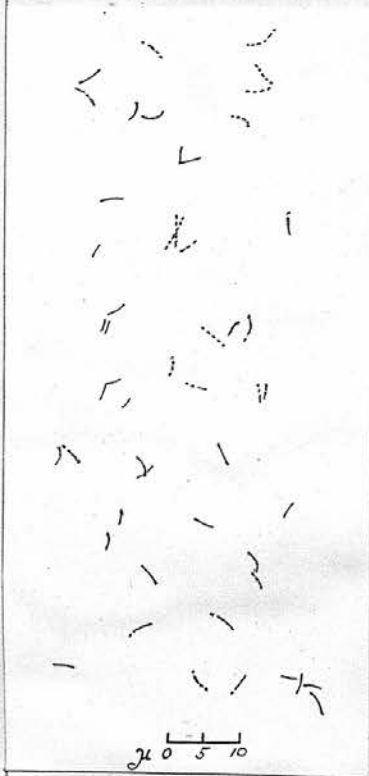
7th Specimen

9. IX. 1913



Ziehl-Nielsen.

Much-Grav.



Ziehl-Neelsen.



Muehler-Gram.

8th specimen 19. IX. 1913.

Mr. J. B. aet 29 years.

Domiciliary treatment at the Royal Victoria Dispensary was begun on 5. V. 1913.

There was marked involvement of the right lung, & considerable systemic disturbance.

Tuberculin injections were begun on 19. V. 1913 (T.B.K.D. 6 cc) & repeated weekly in increasing doses.

There has been a steady improvement in her general condition, & in the affected lung.

Tubercle bacilli were at first very numerous in the sputum, but they have almost disappeared from the now (Nov. 1913) scanty sputum. This has been examined twelve times during six months.



Stain Ziehl-Neelsen.

1st specimen 14. V. 1913.

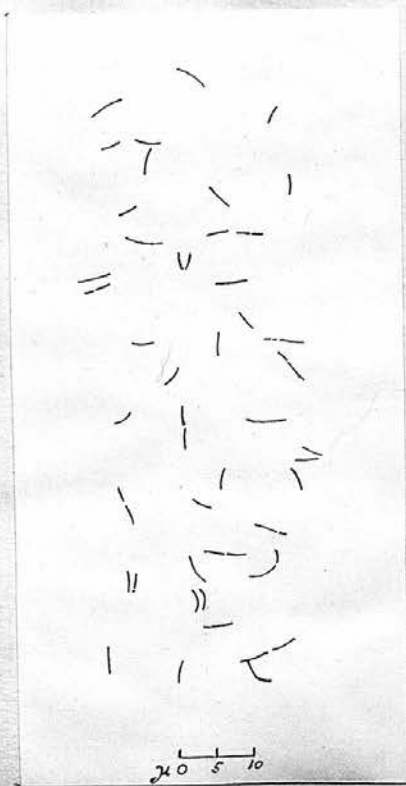
Tuberculin injections not yet begun.



Ziehl-Neelsen.

2nd specimen 23.V.1913.

Tuberculin injections begun on 19.V.1913.



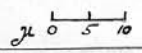
Ziehl-Neelsen.

3rd specimen 29.V.1913.

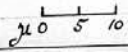


4th specimen

3. VI. 1913.



Ziehl-Neelsen

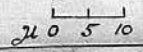


Much-Edaw.

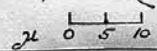


5th specimen

10. VI. 1913.



Ziehl-Neelsen

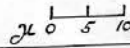
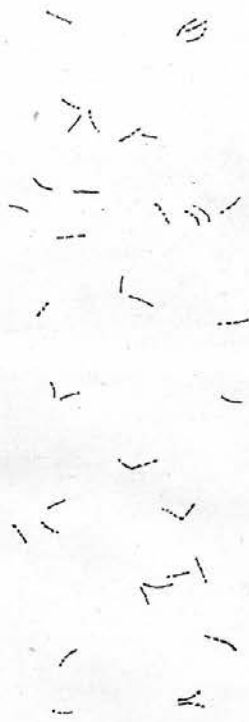
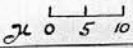


Much-Edaw.



6th specimen

15. VII. 1913.



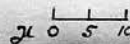
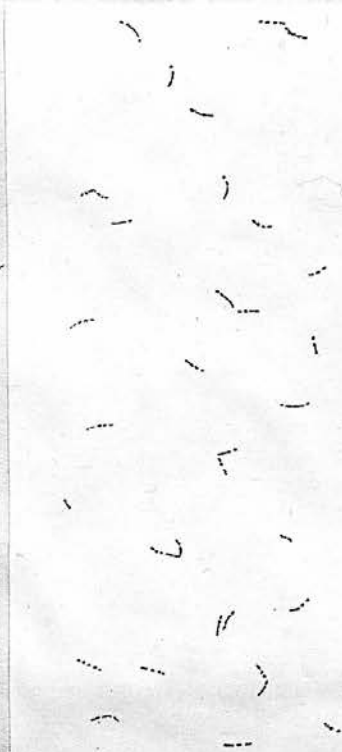
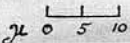
Ziehl-Neelsen

Much-Gram



7th specimen

27. VII. 1913.

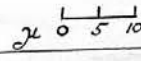
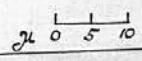


Ziehl-Neelsen

Much-Gram

8th Specimen

11. VIII. 1913

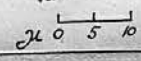
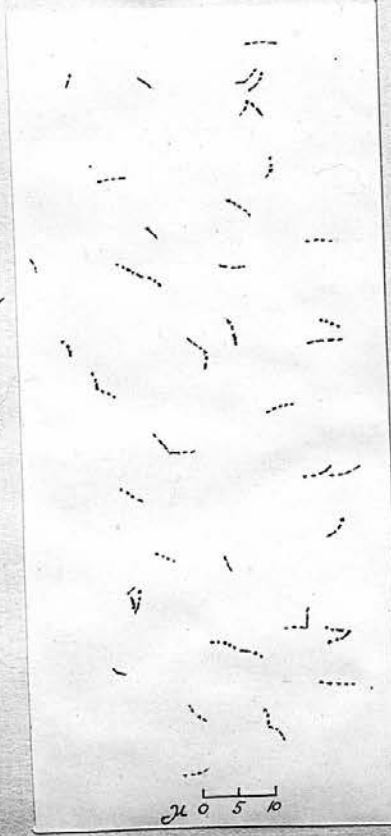
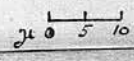


Ziehl-Neelsen.

Much-Gram.

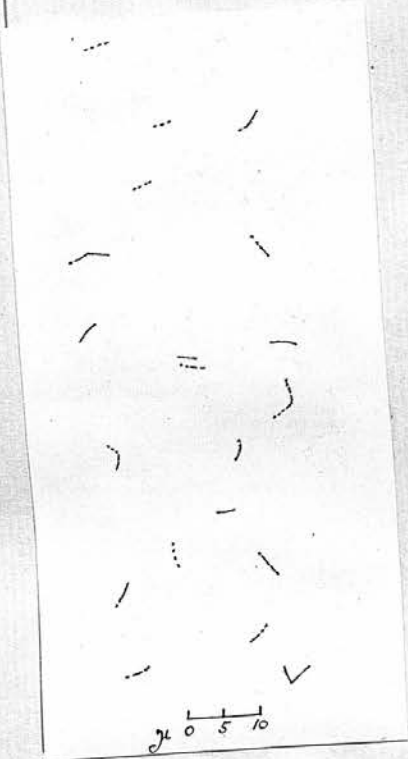
9th Specimen

26. VIII. 1913.



Ziehl-Neelsen.

Much-Gram.



Ziehl-Neelsen.



Much-Gram.

10th Specimen 19.TX.1913.

Jell. male aet. 21 years
Admitted to Royal Victoria Hospital
on 9.V.1913.

Lungs moderately affected, & sys-
temic involvement considerable.

Tuberculin injections were begun on
17.V.1913 (TBKD₆ .5 cc.), the dose being
increased at weekly intervals.

The patient's condition has steadily
improved whilst under observation,
& the bacilli have diminished con-
siderably in numbers from the sputum.
The latter was examined ten times
during six months.



10 5 10



10 5 10

Ziehl-Neelsen

Muck-Lowm

1st specimen 15.V.1913.

Tuberculin injections not yet begun.

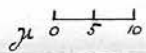
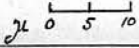
29.



2nd Specimen

3. VI. 1913

Tuberculin
injections
began on
17. V. 1913.



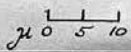
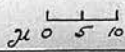
Ziehl-Neelsen.

Much-Gram.



3rd Specimen

20. VI. 1913.



Ziehl-Neelsen

Much-Gram

30.

4th Specimen

1. VII. 1913.



Ziehl-Neelsen.

Much-Gramm.

5th Specimen

8. VII. 1913.



Ziehl-Neelsen.

Much-Gramm.

31.

6th specimen

14. VII. 1913.



Ziehl-Neelsen.

Much-Gram.

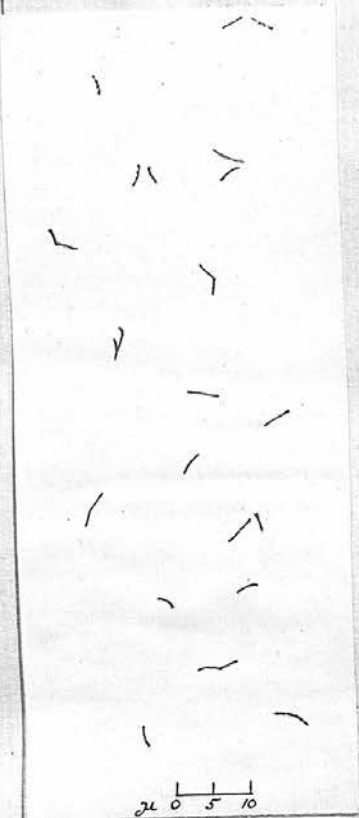
7th specimen

1. VIII. 1913.



Ziehl-Neelsen.

Much-Gram.



Fiehl-Nelsen.



Much-Gram.

8th Specimen 18-TX-1913.

J. P. male aet. 26 years.
Admitted to Royal Victoria Hospital
on 2. V. 1913.

Both lungs moderately affected as
also was the systemic condition.

Tuberculin injections (TBK.D₆ 1cc.) begun
on 20. V. 1913, & continued weekly in
increasing doses up to TBK.D₃ see whilst
under observation.

Lung & general condition improved
during that period, & bacilli were
very scanty in last specimens examined.

The sputum was examined ten
times during five months.



Stain - Ziehl-Neelsen.

1st specimen 15. V. 1913.

Tuberculin injections not yet begun.

34.

2nd specimen

3. VI. 1913

Tuberculin
injections
began on
20. V. 1913

20 5 10

Ziehl-Neelsen

20 5 10

Much-Gran

3rd specimen

20. VI. 1913.

20 5 10

Ziehl-Neelsen

20 5 10

Much-Gran

35.

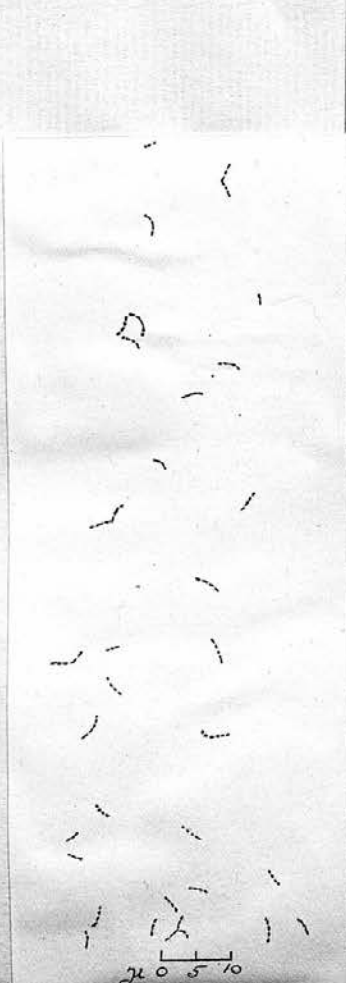
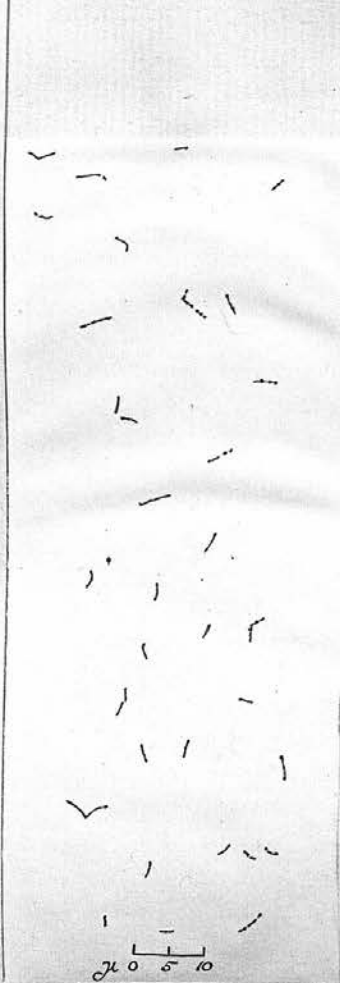
4th specimen

1. VII. 1913.



Ziehl-Neelsen.

Much-Gram.



5th specimen

8. VII. 1913.

Ziehl-Neelsen.

Much-Gram.

36.



6th specimen

15. VII. 1913.



20 5 10

20 5 10

Ziehl-Neelsen

Much-Gran.



7th specimen

1. VIII. 1913.

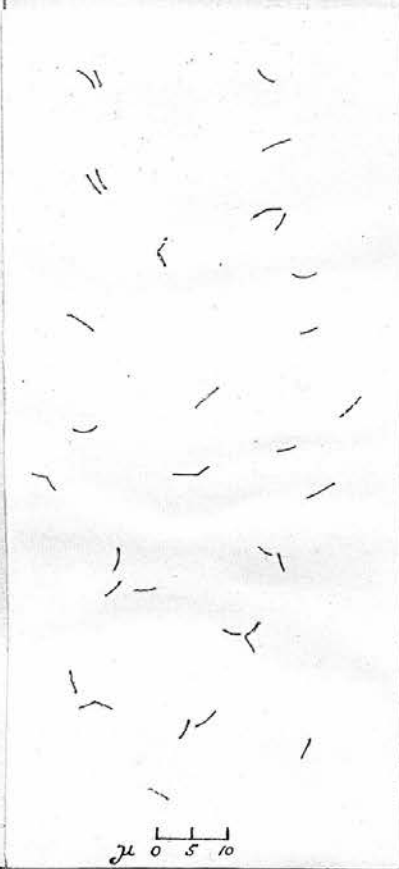


20 5 10

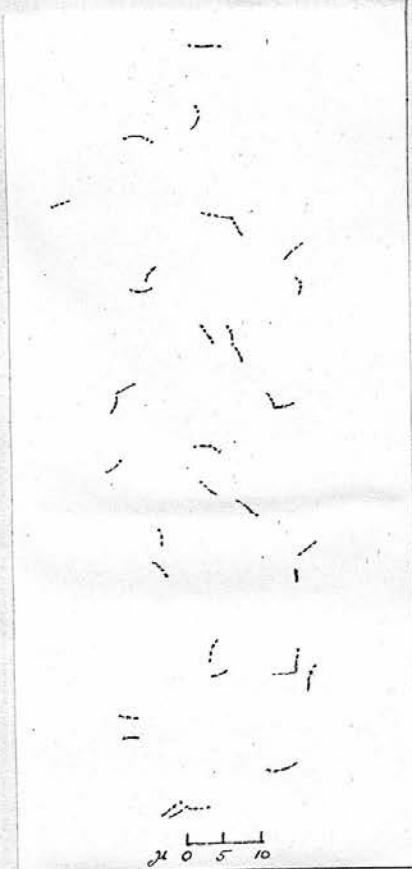
20 5 10

Ziehl-Neelsen

Much-Gran.



Ziehl-Neelsen



Meth-Gram.

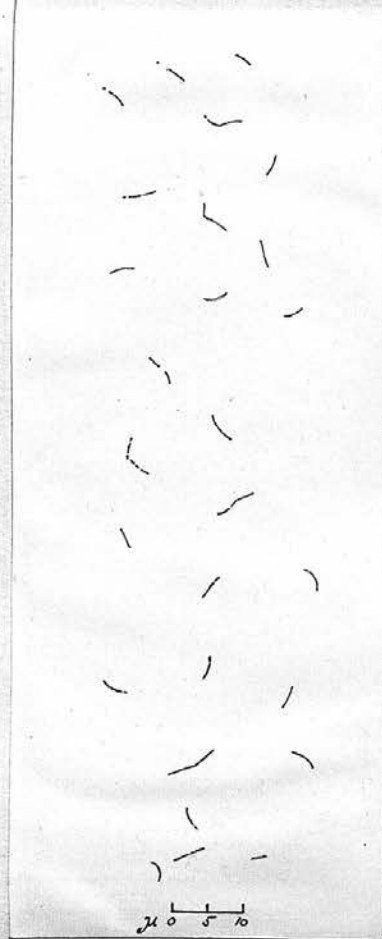
8th specimen 14.VIII.1913.

A.D.M.L. male aet. 27 years.
Admitted to Royal Victoria Hospital
on 25. vii. 1913, with well-marked bi-
lateral pulmonary lesions, & considerable
systemic involvement.

Tuberculin injections (TRK.D₆.5cc) begun on
6. vii. 1913, & repeated weekly in increasing
doses.

Tubercle bacilli were numerous at first
but very few were found in the last
specimens examined.

Twelve specimens of sputum were
examined during five months.



Ziehl-Neelsen.



Much-Gran.

1st specimen 4. vii. 1913.

Tuberculin injections not yet begun.

39.



μ 0 5 10

2nd Specimen

17. VII. 1913

Tuberculin
injections
began on
6. VII. 1913



μ 0 5 10

Ziehl-Neelsen

Muck-Strawm.



μ 0 5 10

3rd Specimen

24. VII. 1913.



μ 0 5 10

Ziehl-Neelsen.

Muck-Strawm.

40.

4th specimen

1. VIII. 1913



μ 0 5 10

Ziehl-Neelsen.



μ 0 5 10

Much-Gram.

5th specimen

8. VIII. 1913



μ 0 5 10

Ziehl-Neelsen.



μ 0 5 10

Much-Gram.

41.



μ 0 5 10

6th specimen

14. VIII 1913



μ 0 5 10

Ziehl-Melzer.

Much-Straw.



μ 0 5 10

7th specimen

22. VIII 1913.



μ 0 5 10

Ziehl-Melzer.

Much-Straw.



Ziehl-Neelsen.



Muehler-Gram.

8th specimen 18. IX. 1913.

M.H. female aet. 31 years.

Admitted to Royal Victoria Hospital on 15.I.1913. There was considerable involvement of the right lung with cavity formation. Systemic disturbance was marked.

Tuberculin injections (TBKD₆ (cc.)) begun on 20.V.1913, & continued weekly in increasing doses up to D₃. see when sputum was last examined.

Whilst under observation the lung condition has improved, as has also the general health.

Bacilli have been considerably reduced in numbers in the sputum. The latter was examined eight times in four months.



Stain Ziehl-Neelsen.

1st specimen 15.V.1913.

Tuberculin injections not yet begun.

44.

2nd specimen

3. VI. 1913

Tuberculin
injections
began on
20. V. 1913.

μ 0 5 10

μ 0 5 10

3rd specimen

20. VI. 1913.

μ 0 5 10

μ 0 5 10

45.

4th specimen

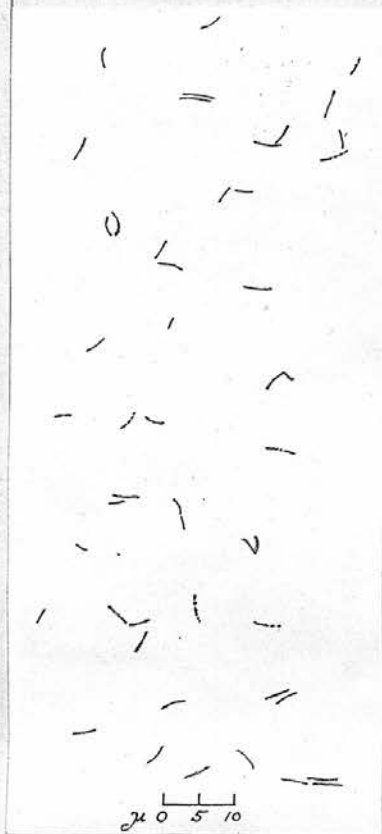
1. VI. 1913.



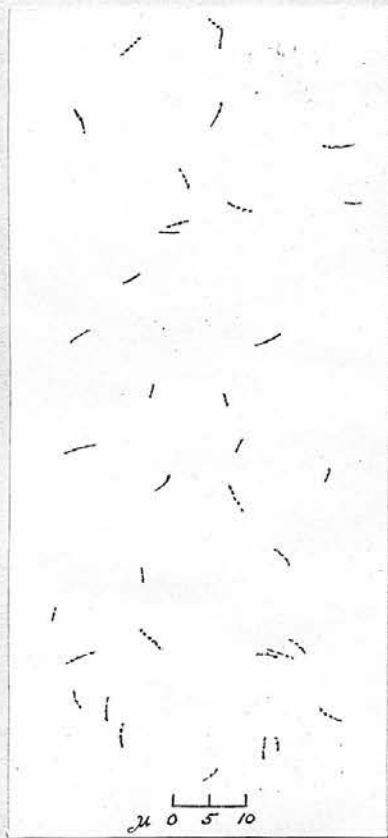
5th specimen

8. VII. 1913.





Ziehl-Neelsen.



Muck-Grans.

6th specimen 14. VII. 1913.

J.B. male aet. 31 years.
Admitted to Royal Victoria Hospital
on 25. vi. 1913, with moderate bilateral
pulmonary lesions, & considerable sys-
temic involvement.

Tuberculin injections (TBKD₆.5cc) begun
on 6. vii. 1913, & continued weekly in in-
creasing doses whilst patient was
under observation.

Improvement has been moderate.
Bacilli increased in numbers in the
sputum after injections were begun,
& diminished latterly.

The sputum was examined eight times
in four months.



Ziehl-Neelsen



Muck-Gram

1st specimen 4 vii. 1913.

Tuberculin injections not yet begun.

48.



2nd Specimen

17. VII. 1913.

Tuberculin
injections
began on
6. VII. 1913.



Ziehl-Neelsen.

Much Gram.



3rd Specimen

1. VIII. 1913.



Ziehl-Neelsen.

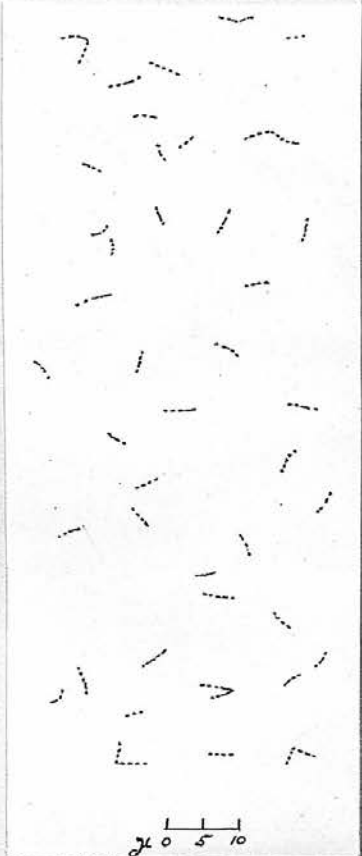
Much Gram.

49.



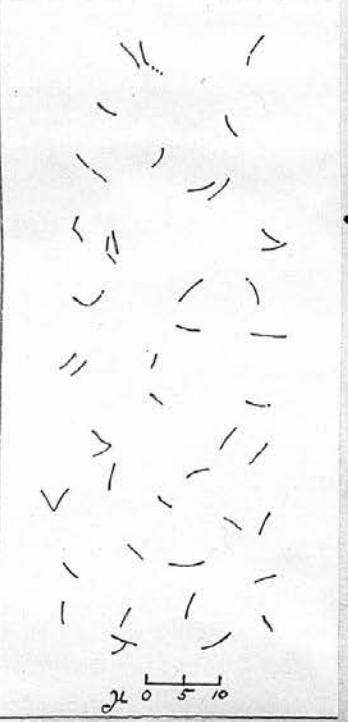
4th Specimen

14. VIII. 1913.



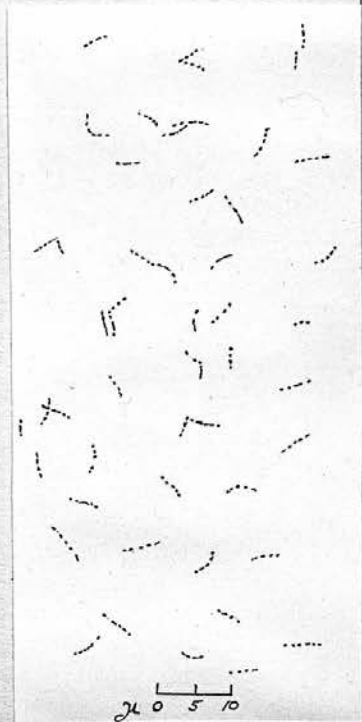
Ziehl-Neelsen.

Much-Gran.



5th Specimen

18. IX. 1913.



Ziehl-Neelsen

Much-Gran.

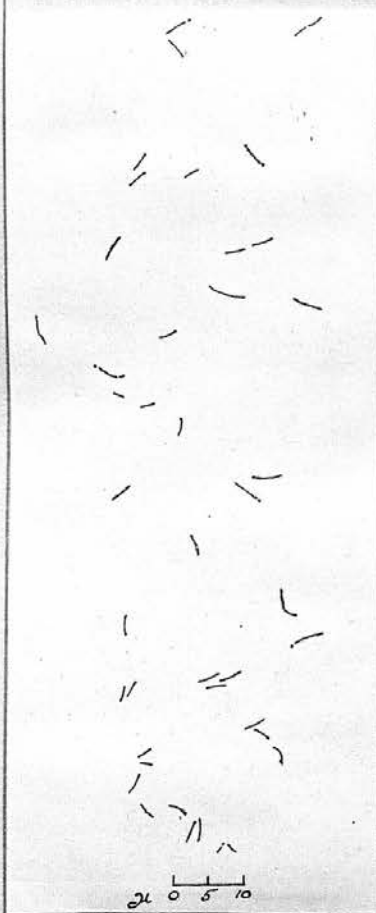
J.A. male aet. 25 years.

Admitted to Royal Victoria Hospital on 21.V.1913. Both lungs were considerably involved, & there was marked systemic intoxication.

Tuberculin injections (TBK D₆ .2 cc) were begun on 10.VIII.1913, & increased at weekly intervals.

The patient has shown considerable improvement. Bacilli were numerous in all specimens examined.

The sputum was examined eight times during four months.



Ziehl-Neelsen



Much Gram.

1st specimen 4.VIII.1913.

Tuberculin injections not yet begun.



μ 0 5 10

2nd Specimen

17. VII. 1913.

Tuberculin
injections

begun on
10. VII. 1913.



μ 0 5 10

Ziehl-Neelsen

Muck-Gran



μ 0 5 10

3rd Specimen

1. VIII. 1913.



μ 0 5 10

Ziehl-Neelsen

Muck-Gran.





Ziehl-Nelson.



Mueh-Grann.

4th Specimen 14. VIII. 1913.

M.C.S. female aet. 37 years.

Admitted to Royal Victoria Hospital on 26.VI.1913, with marked bilateral pulmonary lesions, & considerable systemic involvement.

No tuberculin injections have been given.

Whilst under observation her general & lung conditions have been improving, & the bacilli have been reduced in numbers in the sputum.

The sputum was examined six times in three months.



Ziehl-Neelsen.



Muck-Granm.

1st specimen 14.VII.1913.

No tuberculin injections.

54.



2nd specimen

27. 7. 1913.

No tuberculin
injections.

μ 0 5 10



μ 0 5 10



3rd specimen

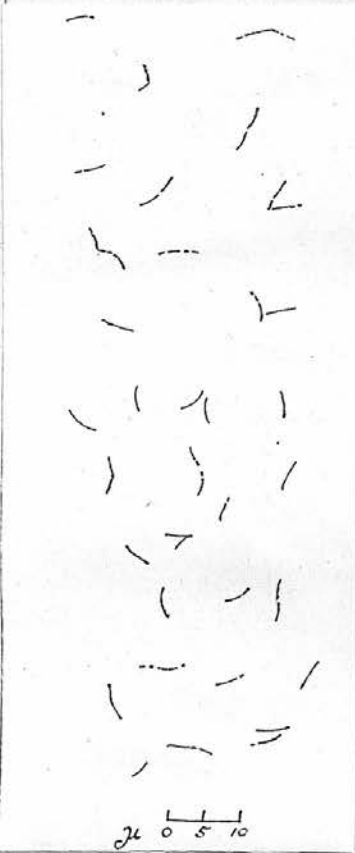
6. 8. 1913.

No tuberculin
injections.

μ 0 5 10



μ 0 5 10



Ziehl-Neelsen



Much-Bram

4th specimen 24.VIII.1913.

No tuberculin injections given.

M^r. M. E. aet. 35 years.

Admitted to Royal Victoria Hospital
on 16. IV. 1913.

Marked involvement of lungs with
cavity formation. Systemic intoxication
marked.

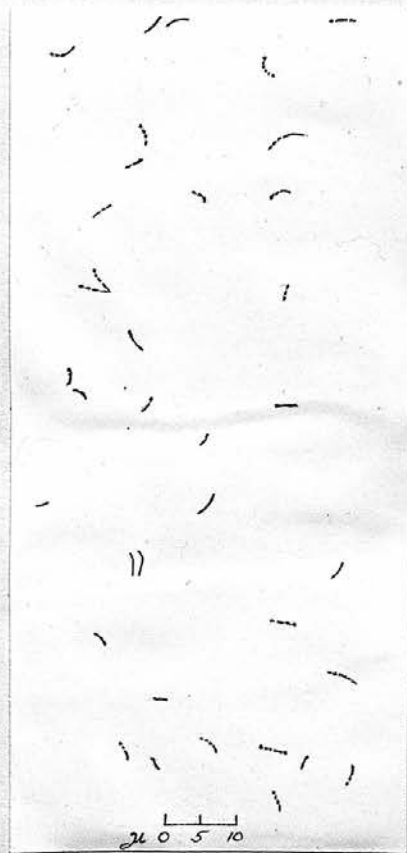
No tuberculin injections have been
given.

Tubercle bacilli have been fairly
numerous in all specimens examined.

The sputum was examined ten
times during five months.



Ziehl-Neelsen.



Muck-Gram.

1st specimen 3. VI. 1913.

No tuberculin injections.

57.

2nd specimen

20. VI. 1913.

No tuberculin
injections

20 5 10

20 5 10

3rd specimen

1. VII. 1913.

No tuberculin
injections.

20 5 10

20 5 10

58.

4th specimen

8. VII. 1913.

No tuberculin
injections.



5th specimen

14. VII. 1913.

No tuberculin
injections.



59.

6th specimen.

1. VIII. 1913.

No tuberculin
injections.



μ 0 5 10



μ 0 5 10

7th specimen

14. VIII. 1913.

No tuberculin
injections.



μ 0 5 10



μ 0 5 10

F.C. male aet. 48 years.

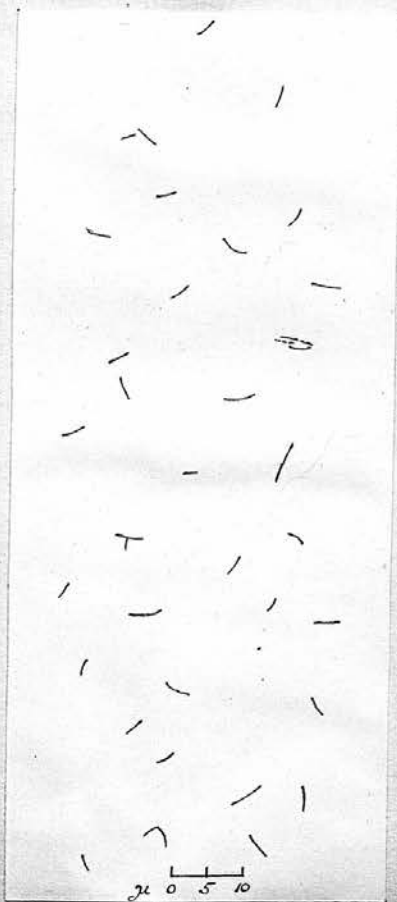
Admitted to Royal Victoria Hospital
on 12. III. 1913.

Both lungs moderately involved, &
there was considerable systemic
intoxication when he was first
admitted.

No tuberculin injections have
been given.

His general condition has improved
considerably. Bacilli were fairly
numerous in all specimens examined.

The sputum was examined eight
times during four months.



Stain - Ziehl-Neelsen.

1st specimen 15. V. 1913.

No tuberculin injections.

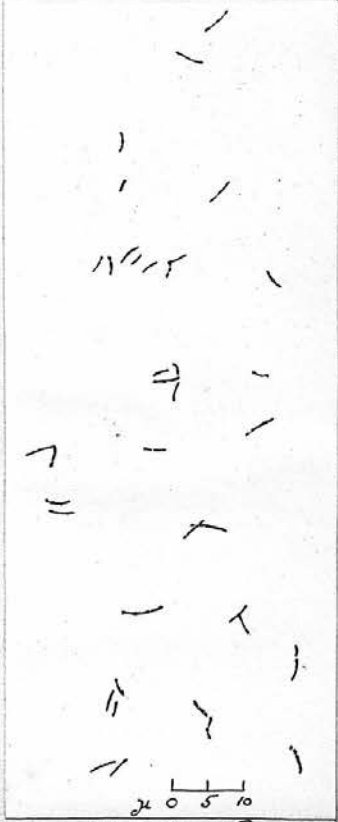
61.



2nd specimen

20. VII. 1913

No tuberculin injections.

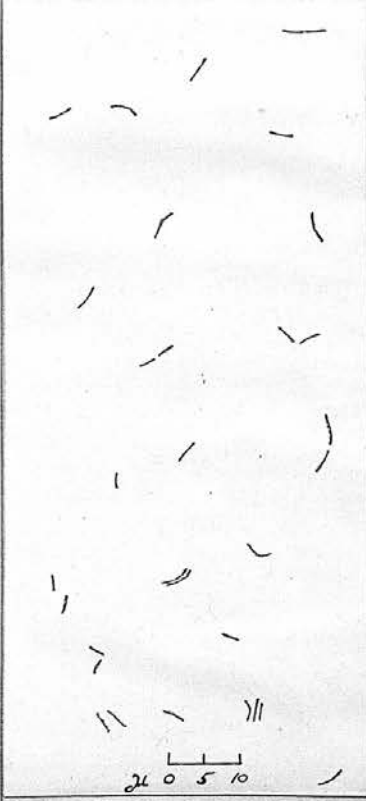


20 5 10

20 5 10

Ziehl-Neelsen.

Muck-Braun



3rd specimen

1. VII. 1913

No tuberculin injections.



20 5 10

20 5 10

Ziehl-Neelsen

Muck-Braun.

62.



4th specimen
8. VII. 1913
No tuberculin
injections.



Ziehl-Neelsen.

Muck-Gram.



5th specimen
14. VII. 1913.
No tuberculin
injections.



Ziehl-Neelsen.

Muck-Gram.

W. J. male aet. 25 years.

Admitted to Royal Victoria Hospital on 8.I.1913, with marked bilateral pulmonary lesions, & considerable systemic involvement.

Tuberculin injections have been given since 15.I.1913 (four months before these observations were begun) & have been continued weekly. Last dose (10.II.1913) was TBKD₂ 1cc.

His pulmonary & general conditions have slowly improved.

Bacilli were found in all specimens examined, but were diminishing in numbers latterly.

The sputum was examined six times in four months.



Stain - Ziehl-Neelsen.

1st specimen 15.V.1913

Tuberculin injections since 15.I.1913.

64.



2nd Specimen

3. VI. 1913.



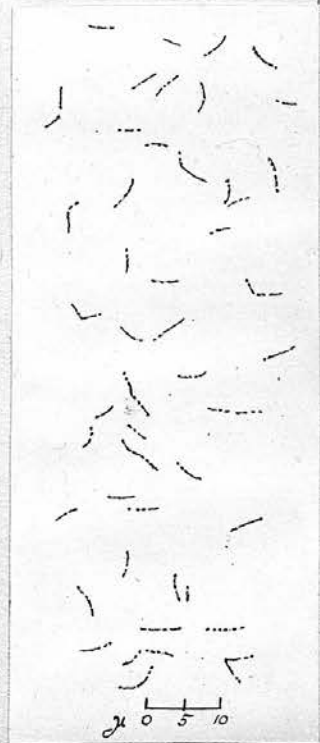
Ziehl-Neelsen.

Much-Gram.



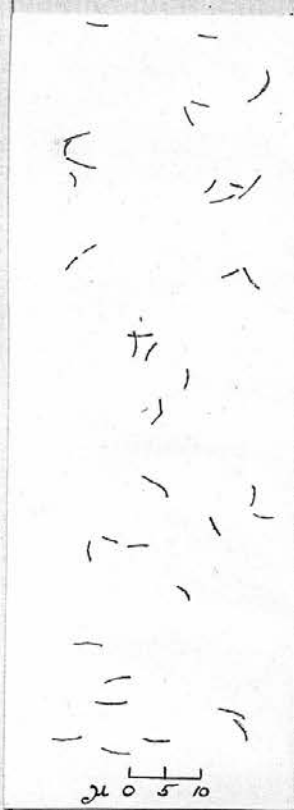
3rd Specimen

20. VI. 1913



Ziehl-Neelsen.

Much-Gram.



4th specimen

1. VII. 1913.



Fiehl-Neelsen.

Muck-Grav.



5th specimen

8. VII. 1913.



Fiehl-Neelsen.

Muck-Grav.

24 0 5 10

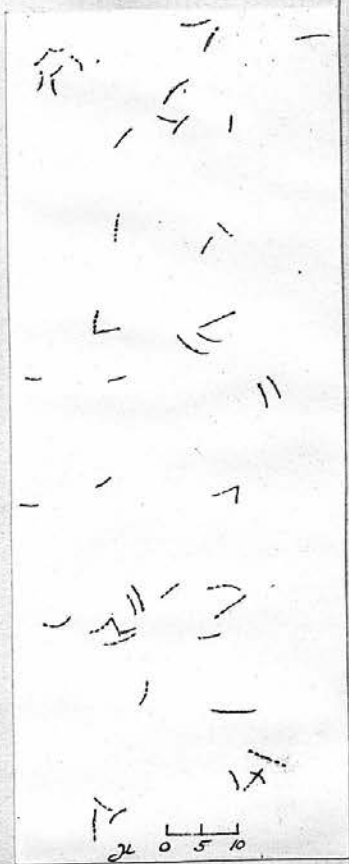
24 0 5 10

24 0 5 10

24 0 5 10



Ziehl-Neelsen.



Much-Gram.

6th specimen 14. VII. 1913.