SYSTEMATIC STUDY
OF THE
GENUS MYCOBACTERIUM

A SYSTEMATIC STUDY OF THE GENUS MYCOBACTERIUM

By

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A Thesis

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I N T R O D U C T I O N

Lehmann and Neumann (54) first proposed the generic name "Mycobacterium" for the group of acid-fast bacteria, with Mycobacterium tuberculosis - the tubercle bacillus of Koch - as the type species. They mention, however, in a second edition of their work, that Metschnikoff (63) had previously proposed for the tubercle bacilli the generic name of Sclerothrix, with Sclerothrix Kochii for the species of tubercle bacillus. They did not know of this, when they first proposed the name Mycobacterium for the genus, and as Metschnikoff had not accurately defined his new genus, according to the rules of botanical nomenclature, they claim their name of Mycobacterium to be still valid.

They describe their genus as follows: "Cultures upon solid nutrient medium are elevated, more or less wrinkled and dry. Microscopically: thin, slender rods, often with typical dichotomous branching, sometimes forming unbranched or branched threads. When the rods have been stained with hot carbol-fuchsin, they give up the stain from the action of acids with great difficulty; they are "acid-proof" - i.e. they behave toward stains much like the spores of ordinary bacilli."

Buchanan (11) mentions the prior use of the generic name Coccothrix by Lutz (56) for the organisms causing leprosy and tuberculosis, and contends that if the acid-fast bacteria are to be recognized as a separate genus the generic designation Coccothrix should be used. He, however, accepts the name Mycobacterium for the genus, stating that inasmuch as the

name Coccothrix has apparently never been used, it may be passed by in the interest of stability.

Chester (17) used the name Mycobacterium to include the group of tubercle bacilli but redefined it so as to also include the genus Corynebacterium of Lehmann and Neumann. His definition reads as follows: "Cells in their ordinary form, short cylindrical rods, often bent and irregularly swollen, clavate or cuneate. At times Y-shaped forms or longer filaments with true branchings. May produce short coccoid elements, perhaps gonidia. Mycobacterium (Lehmann and Neumann) including Corynebacterium (Lehmann and Neumann)." He then subdivides the species of this genus into two groups (1) The non-acid-fast bacilli including the influenza, erysipelas and diphtheria groups and (2) The acid-fast bacilli - the tubercle group.

Castellani and Chalmers (16), 1919, follow Chester in including the diphtheria and glanders types in this genus.

Winslow and the Committee of the Society of American Bacteriologists (97), 1917, Buchanan (11), 1925, and Bergey et al. (7), 1930, adopt the classification of Lehmann and Neumann, separating the acid-fast bacilli into the genus Mycobacterium from the non-acid-fast types, which they place in the genus Corynebacterium.

HISTORICAL

Robert Koch (49), in 1882, was the first to discover the tubercle bacillus and to describe its peculiar staining characteristics and its cultivation on artificial media. His work inaugurated a vast investigation of tuberculosis and the tubercle bacillus, which has resulted in numerous detailed descriptions of the morphological, cultural and biological characters of the organism.

It was Ehrlich (24) who first discovered the peculiar acid-fast staining property of the "Koch bacillus" and the Bacillus leprae, the organism isolated by Hansen (37) and by Neisser (70), in 1879, in the leprous tubercles of persons inflicted with leprosy. This staining property soon led to the discovery of tubercle bacilli or tubercle-like bacilli from lesions in a number of other animals.

Theo. Smith (80), in 1898, was the first to distinguish the acid-fast bacilli in bovine tuberculosis as representing a separate species from the human tubercle bacilli.

Tuberculous infection has been reported among swine, dogs, horses, asses, goats, sheep, cats, rabbits, guinea-pigs, mice, rats and certain wild animals, such as monkeys, lions, tigers, bears, panthers, jaguars, llamas, roes, elephants, when held in captivity. Tubercle bacilli have been isolated from these infected animals, but they all proved to be either of the human or bovine type.

Maffucci (58), in 1890, and Strauss and Gamaleia (86), in 1891, demonstrated the individuality of the "avian" species of tubercle bacillus and carefully described its distinctive characters.

Similar acid-fast tubercle-like bacilli have been isolated and described from tubercles, in a carp by Bataillon, Dubard and Terre (5), in salt water fish by Sutherland (87) and by Aronson (3), in snakes by Sibley (79) and by Aronson (2), in frogs by Kuster (53), in turtles by Friedmann (27), and in a blindworm by Moeller (66).

Whether these acid-fast bacilli are true tubercle bacilli which have undergone slight modifications due to an adaptation to a different environment, or whether they represent separate species of the genus Mycobacterium, is a question over which there has been much controversy. Numerous investigators have claimed to have been successful in transforming one type of tubercle bacillus into another type, but the evidence is by no means conclusive and it is more generally held to-day that these different types of tubercle bacilli represent distinct species in the genus.

Lustgarten (55), in 1884, claimed to have discovered the causal organism of syphilis - an acid-fast tubercle-like bacillus. These results, however, could not be confirmed by other investigators, and it is now generally conceded that the syphilis bacillus of Lustgarten is identical with the Bacillus smegmatis (Mycobacterium smegmatis, Lehmann and Neumann) isolated by Alvarez and Tavel (1) in the smegma of the prepuce and clitoris.

For a while after Ehrlich's discovery of the acid-fast staining property of the tubercle and leprosy bacilli every acid-fast bacillus discovered was considered as a tubercle or lepra bacillus. It was soon found, however, that there were a

large number of acid-fast bacteria closely resembling in morphology the true tubercle bacillus, which were rather widely distributed in nature as saprophytes, incapable of becoming pathogenic. Petri (74), in 1896, was the first to discover these acid-fast saprophytes in butter. In 1898 he published a description of his Butterbacillus (Mycobacterium, Bergey et al.). Rabinowitsch (75) and Korn (51) were also successful in cultivating acid-fast saprophytic bacteria from butter, and Korn in his report, separates the three species, the butterbacillus of Petri, the butterbacillus of Rabinowitsch and his own organism.

Moeller (65 and 66), in 1898, described the "Mist-bacillus", - an acid-fast saprophytic bacterium in dung, - and the "Timothy bacillus", an acid-fast saprophyte on timothy grass. In 1899, he also described another species of acid-fast bacterium on grass - The Grasbacillus 2, (67).

It has been more recently shown that there are a large number of these tubercle-like bacilli distributed in the soil. Kersten (45), in 1909, described an acid-fast and acid-alcohol-fast bacillus in the soil - Mycobacterium alluvialum, Bergey et al. Sohngen (82), in 1913, described six organisms in the genus Mycobacterium, which he isolated from soil as attacking benzene and paraffin compounds. Vierling (91), in 1920, described twenty-three strains of Mycobacteria from the soil, Gray (32) in 1928, isolated one strain - Mycobacterium globerulum - from soil, which decom-

posed indol with the formation of indigotin, and Gray and Thornton (33), in 1928, isolated from soil and described six species of Mycobacteria that decomposed certain aromatic compounds.

Den Dooren de Jong (21), in 1926, described two species of Mycobacteria, - Mycobacterium salmonicolor and Mycobacterium opacum, - which he isolated from water.

It may be mentioned here that these soil Mycobacteria described by Vierling, Sohngen, and Gray and Thornton, and the Mycobacteria of den Dooren de Jong have very doubtful acid-fast properties. Some are described as acid- but not alcohol-fast, some as being acid-fast in young cultures but quickly losing this property with age, and some as being not acid-fast.

If these organisms are definitely non-acid-fast, they should not be placed in the genus Mycobacterium as defined by Lehmann and Neumann and accepted by Bergey and the Society of American Bacteriologists. Bergey (7) in the latest edition of his Manual of Determinative Bacteriology places five of the six of the Mycobacteria of Gray and Thornton (33) among the Actinomyces, but does not classify the organisms described by Sohngen (82) and den Dooren de Jong (21).

Sohngen describes his organisms as all being acid-fast and so should legitimately be placed in the genus Mycobacterium. Vierling (91) describes most of his bacteria as being acid-fast.

The organisms described by Gray and Thornton so closely resemble those described by Sohngen and Vierling, that the

wisdom of separating these organisms into two distinct genera, merely on a staining reaction, which from the investigations seemed very indefinite and variable, is questionable.

In this investigation, it was thought worth while to consider these bacteria as belonging to the genus Mycobacterium for the purpose of studying their relationships culturally and morphologically to the other generally accepted members of the group.

OBJECT OF INVESTIGATION

The object of this investigation, then, is to make a systematic study of the morphological, cultural and physiological characters of a large number of organisms described in the genus Mycobacterium, to show the common relationships of these organisms to each other and to other groups of organisms.

MICRO-ORGANISMS USED IN THIS STUDY

Thirty-one micro-organisms, described by various authors and systematists as Mycobacteria, were studied. (a) The species name, (b) reference to first description, (c) synonym, and (d) source from which each organism was obtained are as follows:-

1. (a) Mycobacterium tuberculosis hominis. (Bergey et al. (7), 1930, p 496-497)

   (b) The "tubercle bacillus", described by R. Koch (49), in 1884.

   (c) Mycobacterium tuberculosis, Lehmann and Neumann (54), 1901, p 410-418.

   (d) Two strains of this organism were studied.

   A:- No. 816 strain, obtained from the American Type Culture Collection, Chicago. Isolated from an East Indian monkey, in which there were intramuscular abscesses and purulent arthritis. Low degree of virulence for monkeys and guinea-pigs. A well-adapted laboratory strain.

   B:- No. 36415 strain, obtained from Dr. Beattie at the Pathological Institute, McGill University. Isolated from a case of acute pulmonary tuberculosis in man.

2. (a) Mycobacterium tuberculosis bovis. (Bergey et al. (7), 1930, p 497-498.)

(b) The bovine type of tubercle bacillus, first described by Theo. Smith (80), 1898.

(d) No. 112 of the American Type Culture Collection.

Note:- A well-adapted laboratory strain. Low degree of virulence for guinea-pigs.

3. (a) Mycobacterium paratuberculosis. (Bergey et al. (7), 1930, p 498).

(b) First isolated and described by Johne and Frothingham (40), in 1895, as the etiological agent of Johne's disease, a chronic diarrhea in bovines.

(d) No. 2531 of the National Collection of Type Cultures, Lister Institute, London.

4. (a) Mycobacterium avium. (Bergey et al. (7), 1930, p 498).

(b) The bacillus of fowl tuberculosis. First described as such by Maffucci (58), and Strauss and Gamaleia (86).

(c) Mycobacterium tuberculosis β avium, Lehmann and Neumann (54), 1901, p 418-420.

(d) No. 116 of the American Type Culture Collection, Chicago.

5. (a) Mycobacterium piscium. (Bergey et al. (7), 1930, p 498-499).

(b) First isolated from tubercles in a carp, and described by Bataillon, Dubard and Terre (5).

(c) Mycobacterium tuberculosis var γ piscicola, Lehmann and Neumann (54), 1901, p 420-421.

(d) No.111 of the American Type Culture Collection.

6. (a) Mycobacterium marinum. (Bergey et al. (7), 1930, p 499).

   (b) First isolated by Aronson (3), from the spleen of a fish, a sargeant-major, (Abudefduff mauritii), and described by him as the cause of spontaneous tuberculosis in salt-water fish.

   (d) No. 925 of the American Type Culture Collection.

7. (a) Mycobacterium ranae.(Bergey et al. (7), 1930, p 499).

   (b) Isolated from the liver of frogs by Kuster (53).

   (d) No. 110 of the American Type Culture Collection.

8. (a) Mycobacterium thamnopheos. (Bergey et al. (7), 1930, p 500).

   (b) First isolated from garter snakes. (Thamnophis sirtalis), and described by Aronson (2), 1925.

   (d) No. 1927 of the National Collection of Type Cultures, Lister Institute, London.

9. (a) Mycobacterium chelonei. (Bergey et al. (7), 1930, p 500-501.

   (b) The "turtle tubercle bacillus", isolated by Friedmann (27), from the lungs of turtles.

   (d) No. 114 of the American Type Culture Collection.

10. (a) Mycobacterium smegmatis. (Bergey et al. (7), 1930, p 501).

    (b) The Bacillus smegmatis of Alvarez and Tavel (1), found on the genitalia, especially in smegma.

    (c) Mycobacterium smegmatis, Lehmann and Neumann (54), 1901, p 424-427.

(d) No. 278 of the American Type Culture Collection.

11. (a) Mycobacterium butyricum. (Bergey et al. (7), 1930, p 501).

(b) First isolated by Petri (74), from butter and described by him in 1897-8. Non-pathogenic.

(d) No. 357 of the American Type Culture Collection.

12. (a) Mycobacterium berolinensis. (Bergey et al. (7), 1930, p 501-502).

(b) The butterbacillus of Rabinowitsch (75).

(c) Mycobacterium lacticola β perrugosum, Lehmann and Neumann (54), 1901, p 431-432.

(d) No. 280 of the American Type Culture Collection.

13. (a) Mycobacterium stercusis. (Bergey et al. (7), 1930, p 502-503).

(b) The "Mist-bacillus" of Moeller (66), 1898, isolated by him from cow manure. Non-pathogenic.

(c) Considered by Lehmann and Neumann (54), 1901, p 433-436, synonymous with Moeller's grass organism I and Petri's butter organism.

(d) No. 77 of the American Type Culture Collection.

14. (a) Mycobacterium phlei. (Bergey et al. (7), 1930, p 503).

(b) The Grass bacillus I of Moeller (65), isolated from Timothy hay, (Phleum pratense). Non-pathogenic.

(c) Mycobacterium phlei of Lehmann and Neumann (54), 1901, p 433-436.

(d) No. 354 of the American Type Culture Collection.

15. (a) Mycobacterium graminis. (Bergey et al. (7), 1930, p 503).

(b) The Grass bacillus II of Moeller (67), isolated

from plant dust. Non-pathogenic.

(c) Mycobacterium lacticola a planus of Lehmann and Neumann (54), 1901, p 429-431. They make a note, however, that: "According to our observations, Moeller's grass organism II is the Mycobacterium phlei."

(d) Obtained from Dr. F. C. Harrison, Pathological Institute, McGill University.

16. (a) Mycobacterium actinomorphum. (Gray and Thornton).

(b) Isolated from soil, and described by Gray and Thornton (33), 1928, p 88-89.

(c) Actinomyces actinomorphus (Bergey et al. (7), 1930, p 471).

(d) Obtained from P.H.H. Gray, Macdonald College.

17. (a) Mycobacterium agreste. (Gray and Thornton).

(b) Isolated from soil and described by Gray and Thornton (33), 1928, p 84-86.

(c) Actinomyces agreste of Bergey et al (7), 1930, p 472-473.

(d) No. 4273 of the American Type Culture Collection.

18. (a) Mycobacterium album. (Sohngen).

(b) Isolated by Sohngen (82), in 1913, from soil, during an investigation of organisms which attack benzene, petroleum and paraffin compounds.

(d) Obtained from P.H.H. Gray, Macdonald College. Original from A.J. Kluyver, Delft, Holland.

19. (a) Mycobacterium coeliacum. (Gray and Thornton).
    (b) Isolated from soil, and described by Gray and Thornton (33), 1928, p 88.
    (c) Flavobacterium coeliacum, Bergey et al (7), 1930, p 156-157.
    (d) No. 4274 of the American Type Culture Collection.
20. (a) Mycobacterium convolutum. (Gray and Thornton).
    (b) Isolated from soil and described by Gray and Thornton (33), 1928, p 87.
    (c) Actinomyces convoluta, Bergey et al. (7), 1930, p 473.
    (d) No. 4274 of the American Type Culture Collection.
21. (a) Mycobacterium crystallophagum (Gray and Thornton).
    (b) Isolated from soil and described by Gray and Thornton (33), 1928, p 86-87.
    (c) Actinomyces crystallophogum, Bergey et al. (7), 1930, p 472.
    (d) Obtained from P.H.H.Gray, Macdonald College.
22. (a) Mycobacterium erythropolis. (Gray and Thornton).
    (b) Isolated from soil by Gray and Thornton (33), and described by them, 1928, p 87-88.
    (c) Actinomyces erythropolis, Bergey et al. (7), 1930, p 472
    (d) No. 4277 of the American Type Culture Collection.
23. (a) Mycobacterium globerulum. (Gray).
    (b) Produces indigotin from indol, isolated from soil and described by Gray (32), 1928.
    (d) Obtained from P.H.H. Gray, Macdonald College.

24. (a) Mycobacterium hyalinum. (Sohngen).

(b) Isolated from soil and described by Sohngen (82), 1913.

(d) No. 617 of the American Type Culture Collection.

25. (a) Mycobacterium lacticola. (Sohngen).

(b) Isolated by Sohngen (82) from soil.

(d) Two strains of this organism were used.

A:- No. 615 of the American Type Culture Collection.

B:- No. 1472 of the National Type Culture Collection, Lister Institute. Dr. St. John-Brooks of the Lister Institute, from whom this organism was obtained, mentions that if this organism is Mycobacterium lacticola a planus of Lehmann and Neumann, it will be the same as Korn's No. I bacillus described by Bergey et al. as Mycobacterium friburgensis.

26. (a) Mycobacterium luteum. (Sohngen).

(b) Isolated from soil and described by Sohngen (82), 1913.

(d) Obtained from P.H.H. Gray, Macdonald College. Original from A.J. Kluyver, Delft, Holland.

27. (a) Mycobacterium opacum. (den Dooren de Jong).

(b) Isolated by L.E. den Dooren de Jong (21), from Maaswater.

(d) Obtained from A.J. Kluyver, Delft, Holland.

28. (a) Mycobacterium rubrum. (Sohngen).

(b) Isolated by Sohngen (82), 1913, from soil.

(d) No. 616 of the American Type Culture Collection.

29. (a) Mycobacterium salmonicolor. (den Dooren de Jong).

(b) Isolated by den Dooren de Jong (82), 1913, from Maaswater.

(c) Flavobacterium salmonicolor Bergey et al. (7), 1930, p 157.

(d) Obtained from A.J. Kluyver, Delft, Holland.

METHODS ADOPTED IN THIS COMPARATIVE STUDY, WITH A BRIEF DISCUSSION OF THE PREVIOUS DESCRIPTIONS OF THE ORGANISMS IN THIS GENUS.

Morphological Characters:-

The shape, arrangement, size, motility, capsule and spore formation, and staining reactions were included in the study of the morphology of the organisms.

Shape, Arrangement and Size. The organisms of this group are described as slender rods, straight or slightly curved. Pleomorphism has been recognized as a very definite characteristic of the group. The tubercle bacillus has been described as assuming a number of shapes and forms; a slender rod, 1.5 to 2.5 μ long, with longer filamentous forms up to 8.0 u long; very small granular bodies, staining non-acid-fast; coccoid bacilli; and even in the virus state. The shape and size of the different pathogenic tubercle bacilli have been used by some investigators to differentiate these types. Theo. Smith (80), from his experiments on the bovine and human types of tubercle bacilli, concludes that the size of the bovine bacillus was quite constant, was usually shorter than the human bacillus and did not tend to change appreciably on prolonged cultivation. The human bacilli were more pleomorphic, showed a tendency to lengthen on artificial cultivation and were more easily changed by certain modifications of the culture medium.

This characteristic is by no means as definite as Theo. Smith concludes. It is the opinion of the majority of investigators who have studied this point, that, although slight differences are noticeable in the morphology of bovine and human cultures, these differences are too small and inconstant to make a sharp or reliable differentiation.

The avian bacilli are recognized by A.S. Griffith (34) as more plemorphic than the mammalian bacilli.

Club-shaped swellings and branched forms have been observed and carefully described by a large number of investigators as occurring amongst almost all of the members of this group.

The tendency to produce long filaments is also a very common characteristic of this group, and the splitting of these long filaments into short coccoid rods or "cocci", as frequently observed more especially among the saprophytic soil forms but also in cultures of the tubercle bacillus, has led the systematists to recognize the close relationship between this group and the Actinomyces. These different forms have been considered by many as involution or degenerative forms, but Kahn (42), Kirchensteins (46) and a number of other investigators have worked out a cycle of development for the tubercle bacillus and its related organisms, showing

the importance of these different forms in the life history of the organisms.

In the present investigation, the shape and arrangement of the organisms were studied in stained preparations from cultures at different ages on slanted beef-peptone agar. Particular attention was paid to pleomorphic forms and representative drawings were made, by means of the Camera Lucida, of the different shapes which these organisms assumed.

The morphology of Mycobacterium paratuberculosis (Johne) which would not grow on plain beef-peptone agar, was studied from slanted 5% glycerol agar.

The size of the organisms was determined by Chinese or India ink preparations from 48 hour old beef-peptone agar cultures. This method was used in preference to the examination of unstained specimens, as by this method clear definition could be obtained without the resultant shrinking or swelling of the cells encountered in the usual staining procedures. In comparing the size of the organisms in a Chinese Ink preparation and in other stained preparations, the cells appeared generally somewhat smaller after staining by Hucker's Gram method and the Acid-Fast stains and, with some organisms,- somewhat larger when stained by the Kopeloff and Beerman Gram technique. The differences, however, are so small that they are relatively unimportant, and as a very marked decrease in size was often noted in the cultures with an increase in the incubation

period, the size has been reported most frequently from the preparations stained by Hucker's method.

The Chinese or India Ink Method:- The bacteria stand out as brilliantly white or colorless bodies on a dark background and may be easily measured with a standardized ocular micrometer.

India ink frequently contains living bacteria, which may cause difficulty in using this material for the study of micro-organisms. Fresh supplies sterilized in the autoclave were used. The technique was as follows:- (See Tanner (89), 1928, p. 78).

1. Secure a tube of dilute India ink and do not shake or disturb.
2. Place a drop in the centre of a clean slide.
3. With a sterile loop or needle, transfer a little growth to the India ink and spread it over an area of 1 or 2 centimeters on the slide, and allow to become thoroughly dry.
4. Mount in Canada balsam.

Motility:- With few exceptions, almost all workers who have studied the organisms in this group have reported them to be non-motile. Schumowski (78), however, claims to have constantly seen a slow motion of the tubercle bacillus, but was unable to observe any flagella either in stained or unstained preparations.

Calmette' (14) states that in very young cultures, the bacilli possess a real motility, having a variable

number of flagella at each pole; these flagella soon became entangled rendering the bacilli non-motile and apparently causing the organisms to adhere to one another to form compact lumps. According to Besson (8); "Ferran says the tubercle bacillus is motile, but the conclusions arrived at in his paper cannot all be accepted unreservedly. Arloing confirms Ferran's opinion. By subcultivating a glycerin potato culture on glycerin broth this observer obtained motile bacilli." The great majority of investigators, however, do not support these views.

All the organisms, however, were again tested for motility. Cultures, 24 hours old, on slanted Glycerol agar were examined in the hanging drop and stained preparations, stained by the Cesares-Gil's Flagella stain as given in the Manual of Methods edited by the Committee of the Society of American Bacteriologists (18), 1926.

Cesares-Gil's Flagella Stain:-

(1)	Mordant.	Tannic Acid	10 g.
		Aluminium chloride (hydrated)	18 g.
		Zinc chloride	10 g.
		Basic Fuchsin	1.5 g.
		Alcohol (60%)	40 c.c.

The solids are dissolved in the alcohol by trituration in a mortar, adding 10 c.c. of the alcohol first, and

the rest slowly. For use dilute with two parts of water, filter off precipitate and collect filtrate on the slide.

(2) Stain - Ziehl's Carbol Fuchsin - (See page 33).

Technique. Place a tiny drop of culture suspension on a scrupulously clean glass slide, which has been heated before use and cooled to about body temperature. When dry, apply the diluted and filtered mordant for 60 seconds. Wash in water. Stain with carbol fuchsin for 5 minutes. Wash and dry.

Capsules.- The literature is not very complete or explicit on the formation of capsules among the members of the genus Mycobacterium. E. O. Jordan (41) states: "A capsular or enveloping substance is produced by tubercle bacilli. It is more abundant in human than in bovine cultures and the amount becomes greater with the length of artificial cultivation on serum." Bergel (6) states that the tubercle bacillus is surrounded by a waxy sheath, and attributes the acid-fastness of the cell to this waxy sheath. Park and Williams (73) with reference to the tubercle bacillus state: "The bacilli have a thin capsule, shown in one way by the fact that they appear thicker when stained with fuchsin than with methylene blue." With the exception of Mycobacterium globerulum (Gray) which is described as having a wide capsule, there seems to be little information regarding definite capsule formation by the other species of the genus.

As a large number of these cultures, however, became very slimy after some weeks incubation, capsule stains were made of all the organisms after 1 to 3 months growth on Glycerol agar.

The methods of Hiss and of Muir for the staining of capsules were tried; the more satisfactory results were obtained with the latter method. The procedure is given below as published by Mackie and McCartney (57), 1928.

Richard Muir's Method:-

(1) Stain: Strong carbol fuchsin

(2) Mordant: Saturated solution of Corrossive Sublimate 2 parts.

Tannic acid (20% solution) 2 parts.

Potassium alum (saturated solution) 5 parts.

(3) Counterstain: - Loeffler's Alkaline Methylene Blue (See page 33).

Technique:- The film is dried and fixed by heat. Stain with carbol fuchsin for 1 minute, with gentle heating. Wash slightly with spirit and then well in water. Mordant for 30 seconds. Wash well with water. Treat with methylated spirit 30 - 40 seconds. Wash in water. Counter-stain with Loeffler's Methylene Blue for 30 - 60 seconds. Wash and dry.

Endospores:- The question of spore formation by the tubercle bacillus is one that has interested and puzzled morphologists since the description of the organism by Koch. The result is

a number of conflicting views, some authors reporting certain structures as spores, others denying the presence of spores. Koch (See Knaysi (48)) originally described the tubercle bacillus as a rod usually curved, and in long forms, showing in unstained, wet preparations two to six shiny (highly refringent) bodies, which do not take up stains and which must be true spores. A.S. Griffith (34) writes: "Sometimes deeply stained globular or oval bodies are seen at the poles or in the length of the bacilli. Both the clear spaces and the darkly stained bodies have been regarded as spores, but it is now generally believed that the former are due to segmentation of the chromophil substance and that the latter are chromatin granules such as are seen in other species of bacteria." Spengler (83) regarded these clear, transparent portions as spores; Metschnikoff (63) as reserve material that does not take up dyes; Grimme (35) and Meyer (64) as fat droplets; while Calmette (14) writes: "We know to-day that they are nothing but small masses of protoplasmic substance having the characteristics of lipoids (The Gram-positive granules of Much)." Besides the colorless structures of Koch, other granules, the deeply stained globular or oval bodies referred to above were considered and are still considered as spores by many investigators. Metschnikoff (63), Nocard and Roux (71), and Marpmann (60) considered these as spores. Lehmann and Neumann (54) state that these latter bodies do not

present the regular form of the true spores of bacilli. Jordan (41) states that they display little or none of the heightened resistance to the action of heat and chemicals which is so characteristic a feature of other bacterial spores. Geo. Knaysi (48) has recently made a very careful study of the morphology of the tubercle bacillus and sums up his findings as follows: "The young cell is surrounded by a very thin elastic membrane, presenting thickened areas and granular appendages over its internal surface. Inside of the membrane is an unusually dense, apparently alveolar cytoplasm permeated by a vacuolar system and containing a series of round or oval granules of various sizes. These granules vary from 2 to 5 or occasionally 6 in number and they are located at definite places in the cell, usually always in the neighborhood of a vacuole. If only two granules are present in the cell, these are usually subpolar; a third granule may occupy the centre of the cell; the rest may be distributed along the long axis of the cell. These granules may multiply by division. In old cells, the membrane becomes thicker, and its granular extensions more conspicuous, while the cytoplasmic granules become less regular in shape and location, and the cytoplasm shows a poverty in material, an aspect of general disorganization and the fine vacuolar system of young cells is lacking.

"The membrane and the granules seem to be made

up of similar substances staining metachromatically with dilute old methylene blue solutions.

"The granules may divide but they do not seem to be associated constantly with cell division.

"The granules are not volutin. They are insoluble in hot water and the acids used and stain deeply with the indifferent fat dyes.

"The granules are not spores, for they are present in the earliest stages of cell development; moreover, they possess no differentiated structure like known spores do."

As the spore stain is essentially the same as the Ziehl-Neelsen acid-fast stain, no other special spore staining technique was employed nor detailed study of the question of spore-formation made.

Staining Reactions:-

As almost all investigators agree on the difficulty of staining the acid-fast bacteria with simple stains, no further work was done on the subject.

The Gram-Stain:- The Gram-stain has been recognized for some time as a very important characteristic in differentiating bacteria. Genera have been differentiated on a basis of their Gram-staining characteristics, for example, the genus Neisseria from the Gram-positive cocci. As is pointed out in the "Manual of Methods" by

the Committee of the Society of American Bacteriologists (18), this reaction is not as clear cut as some of the early workers thought. Many have shown that a large number of organisms are Gram-variable.

All the members of the genus Mycobacterium have been reported as Gram-positive. Gram-positivity, however, is not an absolutely constant characteristic but has been definitely shown to be influenced by factors like age of culture and conditions of environment, and may be entirely upset by treatment with chemical agents. Krylow (52) has shown that very young cultures of tubercle bacilli are not Gram-positive, and it has been demonstrated that the Gram-positivity of Mycobacterium tuberculosis could be reversed by treatment with trichlorethylene.

A. S. Griffith (34) mentions that the tubercle bacillus can be stained by Gram's method, often appearing when stained by this method very granular, resembling a short chain of small cocci, but does not recommend the method.

There are numerous modifications of the original Gram-stain, giving somewhat different results. The two modifications recommended by the Committee in the Manual of Methods as giving the most satisfactory results were used throughout this investigation. These staining procedures are as follows:-

1. Hucker Modification:-

Ammonium Oxalate Crystal Violet:

Solution A.	Crystal Violet (85% dye content)	4.0 grams.
	Ethyl Alcohol (95%)	20.0 c.c.
Solution B.	Ammonium Oxalate	0.8 grams.
	Water	80.0 c.c.
	Mix solutions A and B.	
Lugol's Iodine Solution:	Iodine	1.0 gram.
	Potassium Iodide	2.0 grams.
	Water	300 c.c.
Counterstain:	Safranin (2.5% solution in 95% alcohol)	10 c.c.
	Water	100 c.c.

Technique:- Stain 1 minute with the gentian violet solution; wash in water; immerse in iodine solution for 1 minute; wash in water and blot dry: decolorize in 95% alcohol for 30 seconds with gentle agitation; cover with counterstain for 10 seconds. Wash, dry.

2. The Kopeloff and Beerman Modification:-

Alkaline Methyl Violet:

Solution A.	Methyl violet 6 B	1 gram.
	Water	100 c.c.
Solution B.	Sodium bicarbonate	1 gram.
	Water	20 c.c.

Just before use, mix 30 drops of solution A with 8 drops of solution B.

Iodine Solution:-	Iodine	2 grams.
	Normal solution sodium hydroxide	10 c.c.
Counterstain:-	Basic fuchsin	0.1 gram
	Water	100 c.c.

After the iodine is dissolved make up to 100 c.c. with water.

Technique:- Stain 5 minutes or more with the methyl violet solution; rinse with the iodine solution; add more iodine solution and allow to stand 2 minutes or longer; drain off iodine solution and blot dry (without washing); decolorize with 100% acetone adding drop by drop to the slide while tilted until no color is seen in drippings (generally less than 10 seconds); dry in the air; counterstain 10 - 30 seconds; wash in water and dry.

The organisms were grown on plain beef-peptone agar and incubated at their optimal growth temperatures. Preparations were made from these cultures after 1, 2, 3, 4, 5, 6, 7, 14 and 30 days incubation and stained by both the above-mentioned modifications of the Gram-stain. The relative size, arrangement, and staining peculiarities were noted and the characteristic forms of these organisms were drawn with the aid of the Camera Lucida.

In the case of those organisms - Mycobacterium paratuberculosis, Mycobacterium avium and Mycobacterium tuberculosis hominis (virulent strain from McGill University) -

which did not grow readily on plain beef-peptone agar, preparations were made from glycerol agar slants as soon as there was any visible sign of growth and after 3 months incubation, and stained by Gram.

Acid-Fast Stain.

The acid-fastness of the tubercle bacillus is perhaps the most important laboratory diagnostic characteristic of the organism. Ehrlich (24), 1882, used a stain composed of gentian violet or fuchsin fortified by the presence of anilin dissolved in water. He found that preparations stained by these solutions could not be decolorized by Bismarch brown as in the original method used by Koch; this led him to the use of strong solutions of Nitric acid and so the fundamental acid-fast property was discovered. Since Ehrlich's classic investigation, a great variety of methods for demonstrating this acid-fastness have been proposed. The best known of these are the familiar Ziehl-Neelsen and Gabbet techniques. Besson (8) and Calmette (14) both list about eight to twelve of these different staining procedures.

A number of methods have been devised for distinguishing between true tubercle bacilli and avirulent acid-fast micro-organisms. Most of these depend on the alcohol- or alkali -resistance of the true tubercle bacillus. Besson (8) writes as follows: "Besides the

tubercle bacillus and the bacillus of Veruga peruana, there are a few other bacilli, like the tubercle bacillus, capable when deeply stained of resisting the decolorizing action of dilute acids. Such, for instance, are the smegma bacillus and the bacillus of Tavel (the so-called syphilis bacillus of Lustgarten), but these, unlike the tubercle bacillus are decolorized by absolute alcohol or ether - likewise the various acid-fast bacilli of Bienstock, Gottstein, Moller, Rabinowitsch, Petri, Rubner, Beck, Obermuller, Coggi, etc." In many of the text-books, Mycobacterium smegmatis is distinguished from the tubercle bacillus by its lack of alcohol resistance. Long (95), however, found that the smegma bacilli under prolonged cultivation in the laboratory on glycerin-peptone agar, became practically as acid-fast as tubercle bacilli.

Park and Williams (73) sum up the situation briefly as follows: "Differential staining methods have been devised to separate the non-acid-fast pathogenic bacilli from the tubercle bacillus, and, although in a general way the decolorization by prolonged action of acid and alcohol is presumptive evidence against suspected bacilli being tubercle bacilli, it is an unsafe procedure. Tubercle bacilli vary in their acid-fastness, but the non-pathogenic types vary even more widely, some being extremely resistant to decolorization."

Vierling (91) found that all the organisms in the

genus Mycobacterium isolated by him were acid-fast in the early generations. They were not decolorized with treatment for 15 seconds in 2% Sulphuric acid after staining with heated Carbol-fuchsin; ten of the twenty-three of them, so stained, soon lost this acid-fastness. Three of them resisted decolorization for 1 minute with 2% Sulphuric acid, and only one strain was acid-alcohol-fast.

Sohngen (82) reports that the organisms isolated by him are acid-fast but not acid-alcohol-fast.

Gray and Thornton (33) state that the organisms of the genus Mycobacterium isolated by them are neither acid-alcohol- nor acid-fast.

Many workers have investigated the relationship between age and acid-fastness. Marmorek (59) found that young cultures or the younger portions of old cultures, contained numerous non-acid-fast organisms. Negre, Boquet and Valtis (69) in a study of young films of tubercle bacilli during the first six days of their development on Sauton's medium, found that in a microscopic preparation of one of these films, it was composed in greater part of non-acid-fast bacilli, coloring blue, in the middle of which were disseminated the "Koch" bacilli of which the acid-fastness was more or less pronounced. Wherry (96) refers to the observations of Breskmann (10), who from a tinctorial study of old (16 years) and more recently isolated human cultures

grown on glycerin agar, concluded that the acid-fast property of cultures increases with the duration of growth and that non-acid-fast bacilli occur in greatest numbers in young cultures, though they may also be found in old cultures. Krylow (52) states that very young tubercle bacilli may not stain at all by Ziehl's method, and Suyenaga (88) demonstrated the presence of non-acid-fast bacilli in the edges of young colonies of tubercle bacilli. Kahn (42) traces a cycle of development for the tubercle bacillus and observes a non-acid-fast stage.

Cantacuzene (15) found that young cultures of the Timothy bacillus growing on gelatin, exhibited the same phenomenon. Many non-acid-fast forms were seen in 1, 2 and 3 day cultures, while acid-fastness was general in 15 days.

The relationship between the conditions of environment and acid-fastness has also been studied by many. Wherry (96) was able to render acid-fast saprophytes non-acid-fast by cultural growth under conditions unfavorable to the synthesis of fats. Ferran(25), by a modification of the culture medium (omission of glycerin) or by growth on different media, obtained bacilli which were no longer acid-fast

Some investigators have demonstrated the reverse reaction, making ordinary non-acid-fast bacteria acid-fast by growth on certain media rich in fats.

The organisms under investigation were grown on beef-peptone agar, and preparations made at the end of 1, 2, 3, 4, 5, 6, 7, 14 and 30 days. The preparations were stained by the Ziehl-Neelsen method, to bring out the acid-alcohol-fastness, and by the Ziehl-Gabbet method to discover any differences in acid-fastness. The procedures as given in the Manual of Methods (18) were used. They are as follows:-

1. Ziehl-Neelsen Method.

Carbol-fuchsin:	Solution A.	Basic fuchsin	0.3 g.
		Ethyl alcohol (95%)	10.0 c.c.
	Solution B.	Phenol	5.0 g.
		Distilled water	95.0 c.c.
	Mix solutions A and B.		
Acid alcohol:	Ethyl Alcohol (95%)		97.0 c.c.
	Hydrochloric acid (conc.)		3.0 c.c.
Counterstain:	Loeffler's Methylene Blue		
	Solution A.	Methylene Blue (90% dye)	0.3 g.
		Ethyl alcohol (95%)	30.0 c.c.
	Solution B.	Dilute KOH (0.01% by weight)	100.0 c.c.
	Mix solutions A and B.		

Technique: Carbol-fuchsin with gentle steaming for 3 to 5 minutes; wash in water; decolorize in acid alcohol until only a suggestion of pink remains; wash in water; counterstain with methylene blue 30 - 60 seconds; wash and dry.

2. Ziehl-Gabbet Method.

Stain: Ziehl's Carbol-fuchsin as above.

Gabbet's Solution: Methylene blue (2% by weight) in 25% sulphuric acid (sp. gr. 1.18).

Technique: Carbol-fuchsin with gentle steaming for 3 to 5 minutes; wash in water; decolorize and counter-stain simultaneously with Gabbet's methylene blue 30 seconds; wash and dry.

Cultural Methods.

Culture Media Employed.

The following media have been used to study the cultural, biochemical and physiological characters.

1. Nutrient Gelatin. Bacto-Peptone (Difco), 10 g.; Bacto-Beef Extract (Difco), 3 g.; Bacto-Gelatin (Difco), 180 g.; distilled water, 1000 c.c. Reaction adjusted to pH 7.0.
2. Nutrient Agar. Bacto-Peptone, 5 g.; Bacto-Beef Extract, 3 g.; Bacto-Agar (Difco), 15 g.; distilled water, 1000 c.c. Reaction adjusted to pH 7.0 - 7.2.
3. Dextrose Agar. Nutrient agar with 1% Dextrose added.
4. Glycerin Agar. Nutrient agar with 5% Glycerin added.
5. Dorset's Egg Medium. Prepared according to directions given by Stitt (85), 1927, page 41. Whole eggs are broken into a sterile flask, mixed thoroughly and 25 c.c. water added to every 4 eggs. The mixture is strained through a sterile cloth and tubed in 10 c.c. quantities. The tubes are slanted in an inspissator and kept at

73° C. for 4 or 5 hours on two successive days. On the third day a temperature of 76°C. is applied. Before inoculating, 3 or 4 drops of sterile water were added to each tube.

6. 5% Glycerin Broth. Bacto-Peptone, 5 g.; Bacto-Beef Extract, 3 g.; Glycerin, 50 g.; distilled water, 1000 c.c. Reaction adjusted to pH 7.0 after sterilization.

7. Loeffler's Blood Serum. Dehydrated Bacto-Loeffler's Blood Serum (Difco) was used, prepared according to the standard formula (3 parts Beef Blood Serum and 1 part Dextrose Broth). Coagulation and sterilization was done in the autoclave. The tubes are slanted in the autoclave; the door and all steam outlets are closed and steam is then turned on. Pressure is attained rapidly and held steadily at 15 pounds for 10 minutes, allowing no air or steam to escape. Keeping a constant pressure, the air-steam mixture is replaced with live steam and the tubes sterilized for 20 minutes at 15 pounds pressure. (Reference: "Manual of Dehydrated Culture Media and Reagents" 2nd edition 1929. Digestive Ferments Co. - p 113-114).

8. Glycerin Potato. Prepared according to the method recommended by Giltner (31), p 26-27. Potato plugs are cut, soaked in a dilute (1:500) solution of Sodium carbonate for 30 minutes and then washed in water. The plugs are then soaked in a 20% solution of glycerin for 30 minutes and placed in sterile Roux tubes. 3 c.c. of the glycerin

solution is added to each tube, and the tubes sterilized in the autoclave at 15 pounds pressure for 30 minutes.

9. Litmus Milk and Purple Milk. The dehydrated Difco products "Bacto-Litmus Milk" and "Bacto-Purple Milk" were used. Prepared by dissolving 105 grams of the powder in 1000 c.c. of distilled water, tubing and sterilizing in the autoclave at 15 pounds pressure for 20 minutes. (Reference: "Manual of Dehydrated Culture Media" 1929, p 115-116).

10. Carbohydrate Broth. Peptone, 5 g.; Beef-Extract, 3 g.; and distilled water, 1000 c.c. To this nutrient broth were added the required carbohydrates in 1% concentration. Sucrose, Dextrose, Lactose and Glycerol. 1% of Andrade's indicator was added. The media were sterilized in the autoclave at 15 pounds pressure for 10 minutes.

11. Nitrate Broth. Nutrient Broth, 1000 c.c.; Potassium nitrate, 1.0 g.

12. Nitrate Agar. Nutrient Agar, 1000 c.c.; Potassium nitrate, 1.0 g.

13. Dunham's Peptone Solution (for determination of indol production). Peptone, 10 g.; Sodium chloride, 5 g.; distilled water, 1000 c.c.

14. Starch agar. Bacto-Starch Agar (dehydrated Difco product), which contains Bacto-Beef Extract, 3 parts; Soluble Starch Difco, 10 parts; and Bacto-Agar, 12 parts; was prepared as directed by the Digestive Ferments Co., and 1% peptone added. No adjustment

of the reaction was made.

15. Lead Acetate Agar (to test the production of H_2S). Bacto-Lead Acetate Agar, the dehydrated product of the Digestive Ferments Co. was used. The medium was prepared according to their directions. (Reference: "Manual of Dehydrated Culture Media", 1929, p 101-102).

16. Indol Agar (to test the formation of Indigotin from indol). Nutrient agar, 1000 c.c.; Indol, 0.1 g. Medium sterilized, as usual, in the autoclave at 15 pounds pressure for 20 minutes.

17. Blood Agar. 5 c.c. human blood drawn aseptically into 5 c.c. sterile citrated saline solution (NaCl 0.9%; Sodium citrate 2.0%; distilled water, 1000 c.c.). This mixture is added to 200 c.c. of sterile nutrient agar at 40°- 43°C. and plates poured.

Cultural Characters.

In this study the general growth characters on the media, as above described, were observed.

The gelatin cultures were incubated at 20°C. The other cultures were incubated at the optimal growth temperature of the different organisms.

Gelatin Plates. Colony formation on plain beef-peptone gelatin has been studied only for the non-pathogenic types of this genus, merely because most of them do not grow at 20°C., a temperature necessary for the solidification of the gelatin. Gelatin colonies, however,

have been described for some of the acid-fast saprophytes, such as Mycobacterium phlei, Mycobacterium friburgensis, and Mycobacterium berolinensis, and for the soil forms isolated by Gray and Thornton. The resinous, irregular, pigmented growth is a common character of the group. Only one species, Mycobacterium actinomorphum has been described as liquefying the gelatin.

Microscopic and macroscopic examination of the colonies was made for all the organisms after 10 days incubation. Such features as rapidity of growth, form, elevation, liquefaction and internal structure were observed.

Dextrose Agar Plates. Colony formation amongst the members of this genus has generally been studied on glycerin agar. Some are described as forming dry and crumpled colonies, others as forming smooth and moist ones. All the organisms, with the exception of the typical bovine tubercle bacillus, are described as producing pigment of varying intensity. The filamentous nature of the colonies has been observed by almost all investigators and is characteristic for the group. Gray and Thornton (33) describe long arborescent projections from the edges of some of the colonies. Although the similarity between these organisms and the Actinomyces has been noted by many, in the filamentous

nature of their colonies, the colony of the Mycobacterium differs from the Actinomyces colony in its consistency. Whereas the Actinomyces generally form very cartilaginous-like colonies, which are only broken up with great difficulty, the organisms of this group form colonies not very coherent and easily broken up.

Colony formation was studied on dextrose-beef-peptone agar instead of plain nutrient agar because it was found that all the organisms, with the exception of Mycobacterium paratuberculosis, grew well on dextrose agar, but the growth on plain nutrient agar was sometimes very scanty and slow. Dextrose agar was in many instances, also found superior to glycerin agar.

Microscopic and macroscopic examinations of both the surface and the deep colonies were made after seven days incubation. Any characteristic definite changes in the colony other than that of size or intensity of color, which occurred on further incubation were also noted. In the descriptions, as with the gelatin colonies, special emphasis was given to size of colony, form, surface, elevation, edge, and internal structure.

Agar Tubes. Aronson and Whitney (4), studing the cultural characteristics of 227 strains of tubercle bacilli isolated from guinea-pigs inoculated with suspensions of latent and active tuberculous tissue and non-tuberculous tissue, were able to differentiate the tubercle bacilli of human origin from the bovine

bacilli by growth in 3% glycerin agar, whereas the differences in the degree of growth on Dorset's medium was too inconstant to permit of such differentiation. The human type grew well on the glycerin agar, but the bovine type showed only very scanty growth.

This supports the findings of A.S. Griffith (34) and the Royal Commission on Tuberculosis, who divide the mammalian bacilli into two types, (1) the human type growing well on glycerin agar (eugonic) and (2) the bovine type growing but poorly (disgonic).

It is stressed, however, that these differences are only apparent in the early generations of the cultures.

The color produced by these bacilli on glycerin agar has also been of importance in separating them. The human bacilli are described as forming a yellowish to reddish pigmented growth, whereas the bovine strain produces little or no pigmentation. The avian bacillus, like the human tubercle bacillus, also produces a yellowish pigment on glycerin agar.

The consistency of the growth has served as a specific differentiating character. The tough, coherent type of growth of the mammalian bacilli distinguishes them from the moist, easily emulsified growth of the avian bacilli. F. Griffith (see Besson (8), p 321) states: "On glycerin agar the avian bacillus frequently forms a wrinkled or warty growth resembling a culture of human tubercle bacilli; but the characteristic difference is evident when the growth is

touched with the spatula."

The saprophytic members of the group have all been reported to grow well on glycerin agar producing differing degrees of pigmentation. The raised, rough, often wrinkled or crumpled growth, which gradually spreads over the surface of the agar is characteristic of this group. The resinous, slightly coherent growth, sometimes becoming quite stringy, is also commonly observed amongst the members of this genus.

The growth on slanted dextrose, glycerin, and plain beef-peptone agar was described after forty-eight hours incubation. In those cases, in which no growth or very scanty growth had taken place in forty-eight hours, descriptions were made as soon as visible growth appeared. Any marked changes in the character of the growth, occurring on further incubation were also described. Such features as rapidity of growth, form, elevation, lustre, surface, color, and consistency are stressed in the descriptions.

Dorset's Egg Medium. This medium was specially devised for the cultivation of the tubercle bacilli from pathological conditions. Dorset (23) found that the mammalian bacilli could be divided into two groups on their cultural characteristics on this medium. Group 1-(the bovine type)-showed scanty growth, and in cultures from guinea-pigs, the colonies were flat, small

and tended to coalesce. Group 2 - (the human type) - in original cultures, showed round, elevated colonies and in subcultures the growth was more abundant than in group one, and could not be so easily broken up, being also more adherent to the surface of the culture medium. This medium has, since, been used extensively for the differentiation of the bovine and human bacilli. Wang (93), Griffith (34) and numerous others refer to the differences in growth of the human and bovine bacilli on this medium, the human strain being eugonic and the bovine disgonic.

All the organisms were grown on this medium and descriptions of the cultures made after two weeks growth. Particular attention was given to amount of growth and color, but the other ordinary growth characteristics were also observed. Cultures were also examined for any darkening or digestion of the medium.

Glycerin Broth. The characteristics of the tubercle bacilli of human, bovine and avian origin on glycerin broth have been carefully studied by a number of investigators. Park and Krumwiede (72) in comparing human and bovine bacilli state: "The sharpest separation into two types depending on the amount of growth was made by Kossel, Weber and Heuss and by Oehlecker. They used glycerin bouillon. The human type generally grew well, covering the surface completely in two or three weeks,

climbing on the glass, and forming a uniform thick wrinkled membrane. With the bovine type the growth was much slower and sparser and unreliable. It consisted of a fine net-like or veil-like pellicle and in many cases this was the extent of the growth. In other cases wart-like thickenings occurred of varying diameter, others grew as a uniform spreading pellicle. These differences are present only in freshly isolated cultures."

A.S. Griffith (34), with similar descriptions has confirmed these results, and like Park and Krumwiede, Aronson and Whitney (4) and other investigators, has stressed the fact that these differences in growth can be depended on only when the early generations of the cultures are observed.

The growth of the avian bacilli in broth is somewhat different from that of the mammalian bacilli. Hastings, Halpin and Beach (38), who have made a careful study of this organism, described the growth in broth as at first being confined to the bottom of the flask, later turning the liquid turbid and suddenly forming a thin pellicle which gradually thickens and becomes wrinkled with age. The constancy, however, of pellicle formation by this bacillus has been questioned by some investigators.

Bataillon, Dubard and Terre (5) describe the organism isolated by them from fish (Mycobacterium piscium) as growing well in broth in 3 to 4 days; the

medium never becomes turbid, but there is a granular sediment, somewhat resembling the growth of the avian bacillus.

Topley and Wilson (90) in characterizing the growth of the cold-blooded acid-fast bacilli in glycerin broth state there is no turbidity in the medium, but growth occurs as a thick veil over the bottom of the flask and part way up the sides, made up of coarse interlacing columns; there is no surface growth.

These descriptions are not in accord with A.S. Griffith's (34) findings. He obtains with the bacilli of fish origin a smooth, creamy, easily broken up pellicle in glycerin broth, and describes the bacilli of the cold-blooded animals as growing on the surface of the broth, producing pellicles of varying thickness, which are easily broken and tend to fall to the bottom of the fluid, where they form an abundant flocculent deposit.

Most of the saprophytic forms have been described as forming pellicles of varying thickness and stability. Turbidity sometimes occurs but often the medium remains clear. A sour ammoniacal odor has been observed in the case of Mycobacterium berolinensis (Rabinowitsch) but the majority of cultures remain odorless or have a rather pleasant aroma.

The organisms, during this investigation, were all grown in flasks of 5% glycerin broth, and pellicle formation, the nature and color of the pellicle, turbidity and sediment were observed after 3 days incubation.

Loeffler's Blood Serum. This medium has also been much used in comparative cultural studies of the tubercle bacilli. The eugonic human type has been distinguished from the disgonic bovine type on this medium by numerous investigators. Descriptions have also been given of a large number of the other organisms of this group on blood serum.

Examinations and descriptions of cultures were made after 48 hours growth. With those organisms showing no growth or very scanty growth in 48 hours, the description was delayed until such time as a characteristic growth had occurred. Rapidity of growth, lustre, surface, elevation and color were noted. The cultures were also examined for any darkening or liquefaction of the medium.

Glycerin Potato. This medium has also been of much importance in the isolation and cultivation of the tubercle bacilli. Calmette (14) states that "on this medium the tubercle bacillus grows more rapidly and much more abundantly than on coagulated serum, so that

this medium is very useful for procuring large quantities of bacteria. In four to five weeks the surface of the potato is entirely covered with a thick coating of heaped up granular colonies which stand out irregularly and are of a grayish-white color; at times they are dry or again moist according to the origin of the bacilli, whether human, bovine or avian." Most investigators report a good growth on this medium and have paid particular attention to chromogenesis, as this medium seems especially suitable for pigment production. Vierling (91), however, in his study of Mycobacteria isolated from the soil does not find potato a very good medium for their growth or for pigment production; 5 of his 23 strains did not grow at all on potato and 8 grew but scantily.

The rapidity of growth, form, lustre, and color were the features stressed in the descriptions of the potato cultures. Cultures were described after 48 hours growth, whenever the growth was abundant enough to be characteristic. Any changes, especially in color, occurring with further incubation were also observed.

Gelatin Tubes. None of the genus Mycobacterium with the exception of Mycobacterium actinomorphum has been reported as liquefying gelatin. According to

Gray and Thornton (33), Mycobacterium actinomorphum produced a saccate liquefaction in a nutrient gelatin stab in 8 to 14 days.

The cultural characteristics of the organisms were studied in the gelatin tube. The nature of the growth on the surface and in the depth of the medium was observed, and the absence or presence of liquefaction after 2 months incubation recorded.

Chromogenesis.

Much attention has been given by some investigators - Vierling (91), Sohngen (82), den Dooren de Jong (21) - to the color of the growth of the Mycobacteria, isolated by them, on many varied culture media, and great significance has been attached to it in the differentiation of species. Chromogenesis has also been studied in relation to the other organisms of the group and has formed, in the opinion of some investigators, a real cultural distinction between species.

During this investigation, careful observations were made on the color of the cultures on the media used, and, although marked differences were found occurring with changes in the environment, the colors formed by all the organisms were compared accurately on the same batches of media and after certain definite periods of incubation. Generally Ridgway's (76) color

standards were used as a comparison and the (R) after a color designates a matching of the color of the growth with a particular color in Ridgway's standards.

Biochemical Reactions.

Action upon Milk. The action on milk has been studied for a number of the species of this genus. Some of the organisms have been reported as causing a final alkalinity in the milk; some an acidity; some an acidity and coagulation; some a peptonization; and some a reduction of the litmus (in litmus milk).

All the organisms, during this study, were grown in litmus milk and Brom Cresol Purple Milk, and any changes, such as acidity, alkalinity, reduction of the dye, coagulation and peptonization were observed.

Action on Carbohydrates. There has been very little published on the carbohydrate metabolism of the members of this group. Theo. Smith (81) first showed that mammalian tubercle bacilli could be differentiated into two groups on their action on glycerin broth. One Group - the bovine type - producing alkalinity in 3.5% glycerin broth; the other Group - the human type - producing an initial alkalinity and then a marked acidity in the broth. Since then, much work has been done on the reaction curve in this medium. Although a great many investigators have

been able to obtain these two different curves in glycerin broth, it is the general opinion to-day that the character is too uncertain a one to be used as a definite differentiation between human and bovine tubercle bacilli.

M. Grund (36), Aronson and Whitney (4), and Mohler and Washburn (68) have all obtained so many atypical reactions that they conclude the test to be not specific for the respective types.

Kendall, Day and Walker (43) studying the metabolism of certain saprophytic strains of human tubercle bacilli in plain, dextrose, mannite and glycerin broths observed that there was a progressive alkalinity in the plain, dextrose, and mannite broths, but alkalinity in the glycerin broth was succeeded by acidity. In a later paper the same authors (44) conclude that the "development of an acid reaction in glycerol cultures of human tubercle bacilli and the absence of chemical evidence of proteolysis indicate that the human type can ferment glycerol."

There is evidence from the work of Frouin and Guillaumie (28) that human and avian bacilli can utilize glucose, maltose, saccharose, trehalose and glycerin. Corper and Sweany (20), state that the tubercle bacilli of human and bovine origin do not possess enzymes capable of inverting sucrose, demon-

strable by the Lewis and Benedict picramic acid method.

Weinzirl and Knapton (94) studied the reaction curves of fifteen species of bacteria of the genus Mycobacterium on synthetic media and came to the conclusion that glucose, mannitol, and lactose are apparently not utilized.

Gamble and Herrick (29) have shown by a quantitative method that 5 strains of B. tuberculosis, 2 human, 2 bovine and 1 avian consumed dextrose from a liquid medium.

Merrill (61) found that there was no increase in acidity accompanying the growth of organisms of this genus when grown in carbohydrate broth.

It is quite evident from these results that the Mycobacteria utilize carbohydrates without the production of acids. This was shown by Merril (62), in 1930, who in a study of 13 species of acid-fast members of this genus, came to the conclusion that the method of determining carbohydrate utilization by noting the appearance of acid in the media, which is the method generally used for such bacteriological determinations, is not applicable to the determination of utilization of carbohydrates by organisms of the genus Mycobacterium. He found that the reaction changes in plain broth and carbohydrate broth that typify the organisms of this genus lead toward progressive increase in alkalinity. By direct quantitative carbohydrate determinations, however, using the Shaffer-Hartmann blood sugar quantitative method, he showed

there was a rather wide utilization of carbohydrates by the organisms studied.

Gray and Thornton (33) studied the action of the Mycobacteria isolated by them on glucose, sucrose, lactose, maltose and glycerin by the "acid" production method. Of 74 strains of Mycobacterium agreste, 19 fermented dextrose with acid and 21 glycerin; none of the other species fermented any of the carbohydrates used. Sohngen (82) describes the organisms isolated by him as assimilating a number of sugars. Vierling (91) noted the production of an invertase by 2 of his 23 strains of Mycobacteria.

No quantitative determinations on sugar utilization was made in this study. Any Acid formation by the organisms in one per cent carbohydrate broths (lactose, sucrose, glucose and glycerin) with Andrade's indicator was noted.

The final reaction in 5% glycerin broth, adjusted to pH 7.0 after sterilization, was also observed after 4 weeks incubation, using the LaMotte Hydrogen-Ion Colorimetric Determination apparatus.

Action on Nitrates. The reduction of nitrates to nitrites has formed an important differential test amongst certain types of bacteria. Data, however, with regard to the reduction of nitrates by the members of this genus are very meager. A number of the soil forms

have been reported as reducing nitrates, but in the published descriptions of the pathogenic forms the presence or absence of this character has not been recorded. Vierling (91) reported that 13 of his 23 strains reduced nitrates. Sohngen (82) states that the strains isolated by him do not produce gas from nitrates, but form a little nitrite. Gray and Thornton (33) observed the reduction of nitrates to nitrites by three of the species isolated by them.

As this test seemed to have a fair degree of definiteness, it was included in this study. The organisms were grown in tubes of Nitrate Broth and slanted Nitrate Agar. The cultures were tested after 1, 2, 4, 7 and 10 days growth for the presence of nitrites. As the evolution of gas in a nitrate medium containing no sugar or other similar source of gas is a definite indication of"denitrification" or a complete reduction of the nitrate, the tubes were also observed for gas formation. The reagents used to test for the presence of nitrites were sulphanilic acid and alpha-naphthylamine as recommended by the Committee of the Society of American Bacteriologists in the Manual of Methods (18), page C 28.

Production of Indol. A few of the members of this genus have been reported as producing Indol from peptone.

Rabinowitsch (75) obtained a trace of indol in her cultures of Mycobacterium berolinensis. Petri (see Korn (51)) observed a slight formation of indol by Mycobacterium butyricum and Korn obtained indol formation in glycerin bouillon by Mycobacterium friburgensis. Mycobacterium alluvialum is also described as forming indol from peptone.

The organisms, under investigation, were grown in tubes of 1% peptone water and were tested for the presence of indol after 5, 10 and 20 days incubation by the Ehrlich-Bohme technique, as given in the Manual of Methods (18), page C 31, using di-methyl-amino-para-benzaldehyde dissolved in ethyl alcohol and concentrated HCl, and a saturated aqueous solution of potassium persulphate ($K_2S_2O_3$).

The Hydrolysis of Starch. As with the nitrate reduction test, this character has been of importance in differentiating certain types of bacteria, especially soil forms. It has, as a rule, played little part in the study of pathogenic bacteria, and there is little mention in the literature of the action of the pathogenic members of this genus on starch. Corper and Sweany (20) state that the tubercle bacilli of both the human and bovine varieties, or autolysates therefrom do not possess enzymes capable of hydrolyzing starch, demonstrable by the delicate Lewis and Benedict

picramic acid method. Sohngen (82), Vierling (91), Gray and Thornton (33), however, have observed the secretion of a "diastase" enzyme by a number of the saprophytic Mycobacteria.

To study the action of the organisms on starch, plates of starch agar were prepared and the organisms streaked over the surface of the Agar. After 2, 7 and 14 days incubation, the cultures were tested for starch hydrolysis by flooding the plate with a saturated solution of iodine in 50% alcohol. A clear zone around the area of growth indicated hydrolysis.

The Production of Hydrogen Sulphide. This test has been of importance in separating certain species of the intestinal group of bacteria, but has played little part in any systematic studies of the members of the genus Mycobacterium. With the exception of Mycobacterium berolinensis and Mycobacterium alluvialium, none of the other Mycobacteria has been described as producing H_2S.

The production of H_2S was tested for by growth in tubes of Lead acetate agar. Slanted agar tubes were inoculated by streaking along the slanted surface and stabbing at the base of the slant through the condensation water. The production of H_2S causes a blackening or browning of the medium along the line of inoculation.

Physiological Characters.

Relation to Free Oxygen. All the members of this genus have been reported as strongly aerobic and a few as facultatively anaerobic. No study was made on the oxygen requirements of the organisms in this investigation, other than a preliminary growth in Dextrose agar in a Burri tube, from which the oxygen was removed by pyrogallol and sodium hydroxide.

Relation to Temperature. The genus Mycobacterium includes organisms with widely different temperature requirements. The avian tubercle bacillus has a somewhat higher optimum temperature for growth than the mammalian bacilli, while the bacilli of frogs, fish, turtles, snakes and other cold-blooded animals have a still lower optimum growth temperature. The soil forms are reported to thrive best at temperatures ranging from 25°C. to 35°C.

The temperature range for growth reported for most of the pathogenic forms is very narrow. Buchanan and Fulmer (12) state that for Mycobacterium tuberculosis hominis it is only 8°C. to 10°C. Bulloch (13) gives the minimum and maximum temperatures for growth of the tubercle bacillus as 29°C. and 42°C. Schlossberger and Pfannenstiel (77) in an attempt to differentiate strains of genuine tubercle bacilli from non-pathogenic, morphologically similar acid-fast bacilli, state that the

optimal temperatures and the temperature limits of growth sharply differentiated the two groups. The true tubercle bacilli (with the exception of the "atypical" forms, such as described by Arloing) do not grow at a temperature above 42°C., while the non-pathogenic acid-fast bacilli are able to grow at 50°C. and some at 55°C. and 58°C. Genuine tubercle bacilli of cold-blooded animals do not flourish at 37°C.

Topley and Wilson (90) state that the human, bovine and avian types do not grow below 30°C., while the cold-blooded and saprophytic acid-fast types grow freely at 20°C.

Stableforth (84) gives for Mycobacterium para-tuberculosis an optimum temperature of 39°C. to 42°C. with limits of growth 30°C. and 43°C.

Vierling (91) states that with the strains, isolated by him, growth occurs between 3°C. and 38°C. Under 20°C. growth is slow, best as 32°C. and stops over 38°C.

Atypical strains, however, have been described, the temperature limits of which are much wider than those reported for the typical organisms.

In reporting minimal, optimal and maxinal temperatures for growth of organisms, Buchanan and Fulmer (12) stress the importance of the environment

on the temperature relations. "The error" they write "is not infrequently committed, of speaking of the optimum temperature for the growth of a particular organism. Apparently each organism may have an indefinite number of optimum temperatures depending upon the various other environmental influences." And a little further on they emphasize the fact that the minimum and maximum growth temperatures are not to be regarded as fixed temperatures. Just as with the optimum, they may vary within relatively wide limits with variation in environment.

To determine the temperature relationships of the organisms, under investigation, a light suspension of each organism was prepared in sterile physiological saline solution, and one loopful streaked across the surface of a dextrose agar slant or glycerin agar slant (in the case of Mycobacterium tuberculosis hominis (McGill strain) and Mycobacterium avium, which grew more easily on glycerin than on dextrose agar). A number of these slant cultures were prepared and incubated in chambers at temperatures graded from 0°C. to 60°C. The temperature at which the tubes showed the first signs of growth was recorded as the optimum growth temperature, and the lowest and highest temperatures at which growth appeared after prolonged incubation were reported as the minimum and maximum growth temperatures respectively.

The Relation of the Reaction of the Medium to Growth.

Although the reaction of the medium is one of the most important factors influencing the growth of micro-organisms, the effect of pH on the growth of the Mycobacteria has been little studied. Kondo (50), Dernby (22), Ishimori (39), and Gieszczkyiewicz and Wroblewski (30) have reported minimal, optimal and maximal Hydrogen-ion concentrations for the growth of the tubercle bacillus, but their results do not agree. These results may be compared:-

	Minimum	Optimum	Maximum
According to:			
Kondo (50)	5.0		8.4
Dernby (22)	6.0	6.8-7.2	7.6
Gieszczkyiewicz and Wroblewski (30)	4.0	7.3-7.7	7.9
Ishimori (39)	6.6	7.4-8.0	9.5

Martin Ficker (26), however, in a comparative cultural study of the tubercle bacillus on a variety of acid, amphoteric, neutral and alkaline media found that the organisms grew better on the acid or amphoteric substrates than on the neutral or alkaline ones.

M. Brooks (9) found that changes in the pH of the medium from pH 4.4 to 7.4 had no effect on the rate of production of carbon dioxide by the tubercle bacillus. When the acidity is increased beyong pH 4.4 the rate of production of CO_2 is decreased but becomes constant and remains so for a long time.

When the alkalinity is increased beyond pH 7.4 the rate of production of CO_2 is increased, but subsequently returns to the normal.

With regard to the other members of the group, Ishimori (39) reports the following pH values for growth in 4% glycerin bouillon:

	Range for Growth	Optimum
M. berolinensis	4.7-11.1	5.7-8.5
M. phlei	5.7-11.2	7.5-9.1
M. graminis	6.2-10.5	7.4-7.7
M. chelonei	6.5-10.1	7.5-7.7
M. ranae	6.0-10.3	6.2-7.5
M. tuberculosis (bovis)	5.2- 8.7	5.8-6.9

Kondo (50) gives the range for Mycobacterium phlei as pH 5.0 - 8.4. Gieszczkyiewicz and Wroblewski (30) using 7 strains of tubercle bacilli of human origin, 3 of bovine origin, 1 avian bacillus, and 1 bile-treated bacillus (B.C.G.) in 4% glycerin broth found that some developed at pH 4.0 but the greater number were stopped in their growth at pH 5.0. None could grow at pH 8.0 but certain strains starting in a medium less alkaline than pH 8.0 could grow and render the medium as alkaline as pH 8.4 (in the case of the human bacilli) and pH 8.6 (with the avian bacillus). The optimum for most of them was between pH 7.3 and 7.7. There was no difference between the human, bovine and avian strains.

As the observations on the pH relations of this group are so scanty and contradictory, it was decided to observe the minimal, optimal and maximal pH values for growth of all the organisms on 1% dextrose agar and 4% glycerin broth.

Because of the marked effect of sterilization on the Hydrogen-ion concentration of a medium, the reaction was always adjusted after sterilization with sterile normal HCl and normal NaOH. The pH values of the media, between pH 3.5 and 8.0 were obtained with the quinhydrone electrode. For media more alkaline than pH 8.0, indicators were used to determine the reaction.

It must be mentioned here that the pH values in the case of the agar media are not absolutely accurate, because deter-

minations had to be made while the agar was warm, to prevent its solidification, and it has been quite definitely shown that the pH of a medium when warm is not exactly the same as the pH of the same medium when cool.

The agar was slanted in sterile tubes, so that no further heat was applied after the reaction had been adjusted, and the glycerin broth placed in sterile flasks. The pH of the medium in which growth first appeared was recorded as the optimum pH value for growth, and the lowest and highest values, at which growth took place after a prolonged incubation, were also observed and reported as the maximum and minimum values, respectively.

The Production of Indigotin. Gray (32) described, in 1928, an organism, Mycobacterium globerulum, isolated from soil, which is capable of decomposing indol with the formation of blue crystals. These blue crystals proved to be indigotin. Since the publication of this paper, A.J. Kluyver (47), using a strain of Mycobacterium phlei (Sohngen), obtained with this organism a similar production of indigotin from indol.

This test was used, during this investigation, to discover whether indigotin production from indol was a common characteristic of the group.

Indol agar plates were poured to study colony formation. The production of bluish colonies or crystals

extending out into the medium around a colony, was recorded as the production of "indigotin".

The Action on Blood. Waksman (92) has found that a number of the Actinomycetes are capable of haemolyzing blood and has differentiated species on the absence or presence of this characteristic. There is no mention in the literature of haemolysis of blood by any of the Mycobacteria, but as a number of the organisms, in this study are soil forms and bear a close similarity to the actinomycetes, it was thought worth while to study the action of all of the organisms on blood.

The blood agar plate was used, and the organisms streaked over the surface. The surface inoculations were so made that single colonies could be observed as well as a streak culture. The production of a green zone was noted, and a distinct clearing around a colony was recorded as haemolysis.

DESCRIPTION OF SPECIES

1. A. Mycobacterium tuberculosis hominis

I. Morphology. (Studied from B.P. Agar cultures at 37°C.).

Rods, ranging in size from 1.5 to 8.0 μ in length by 0.2 to 0.5 μ wide. Majority 0.35 μ by 3.5 μ. Curved and occasionally showing some long filamentous forms in older cultures (1 month). Organisms get smaller with age; they are much longer in a 24-hour culture than in a 48-hour old one, but they retain their shape and size after 48 hours growth. Occur singly, in short chains and often show a parallelism in arrangement. No branched forms were observed. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Very uneven staining. Gram-positive granules or segments in a pale blue or pink cell give a very beaded appearance (Pl. 1., figs. 1 and 2.).

(Hucker). 24 hours. Gram-positive. In some cells, more deeply staining granules situated at the end or ends of the rods, make them appear definitely club-shaped. In older cultures, the organisms stain more weakly by Gram and appear more granular. (Pl. 1., figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). The organisms stain almost solidly red, with only a few cells staining irregularly and showing darkly-staining granules in

a pale blue cell. (Pl. 1., figs. 5 and 6). (Gabbet). As with Ziehl-Neelsen's technique (Pl. 1, figs. 7 and 8).

Muir's Capsule Stain.(from Glycerin Agar cultures - 3 months). The individual cells show a very narrow enveloping capsule. Long strands and masses of gummy material, in which the bacilli are held grouped together, are observed.

II. Cultural Characters. (at 37°C.).

Gelatin colonies.(10 days at 20°C.). Very poor development. Minute colonies, becoming in 4 weeks about 1 m.m. in diameter. Very irregular. Surface raised and segmented. Internal structure (magnified), a mass of closely packed filaments. No liquefaction.

Dextrose Agar colonies. (7 days).

Surface colonies. Good growth, from 1.5 to 3.0 m.m. in diameter; irregular; rough and ridged; raised; edge uneven and lobate; internal structure (on magnification) filamentous.

Deep colonies. Much smaller. Filamentous.

Glycerin Agar Stroke.(48 hours). Growth abundant; spreading; raised; at first moist turning dry; slightly wrinkled; grayish-white. Becoming, in 9 days, very wrinkled, pale orange yellow (R), and somewhat stringy, not easily emulsifiable.(

Dextrose Agar Stroke. (48 hours). Rapid, abundant growth; spreading; very slightly raised, membranous-looking; moist; slightly wrinkled; pale yellow (R). Becoming(4 weeks) folded and very stringy.(Pl. 27,fig.1.A).

Nutrient Agar Stroke. Growth similar but less rapid and abundant than on Dextrose or Glycerin.

Dorset's Egg Medium. (2 weeks). Good growth; spreading; raised; moist; granular; light ochraceous buff (R). Medium not darkened or digested.

Glycerin Broth.(3 days in flasks). Clear, with isolated flaky pellicle growth, which in time covers the surface of the medium and grows part way up the sides of the flask, becoming slightly wrinkled. (7 days). A flaky membranous sediment settles to the bottom of the flask, and the pellicle becomes thicker and more deeply wrinkled. (Pl.31, fig. 2.A).

Loeffler's Blood Serum. (48 hours). Good growth; spreading; very moist; smooth but lumpy; grayish becoming in 7 days pale ochraceous buff (R). No digestion or liquefaction of the medium.

Glycerin Potato. (48 hours). Good growth; filiform; raised; dry; smooth; light drab (R). Becoming (3 weeks), thick, spreading, very wrinkled, and orange buff (R).

Gelatin Stab. Grayish, raised, moist surface growth. Very scanty, transparent, filmy growth along line of inoculation. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Whitish pellicle growth. Alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from. Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks) pH 6.8.

Nitrate Broth and Agar. Nitrates reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. No growth in the medium. Surface growth turned brown. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature (Dextrose Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth	52°C.
Minimum temperature for growth	15°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.6 - 7.3.

Limits of pH for growth; from 4.4 to 10.0.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

1. B. Mycobacterium tuberculosis hominis.

I. Morphology. (Studied from Glycerin Agar cultures at 37°C.).

Rods, varying in size from 1.5 to 6.0 μ in length by 0.2 to 0.5 μ in width. Majority about 3 to 3.5 μ long. Highly curved with a few branching forms. The cells become slightly smaller with age. The bacilli generally lie singly or in tangled clumps. Non-motile. No spores formed.

Staining Reactions.

Age does not seem to affect appreciably the staining properties of this organism. The cells appear very beaded and granular by almost all the staining procedures used. The staining reactions are very similar to those of species 1 A. (See Pl. 2, figs. 1 and 8).

II. Cultural Characters. (at 37°C.).

Gelatin colonies. No growth at 20°C.

Dextrose Agar colonies. (4 weeks). Very small; irregular; crumb-like; raised; edge uneven; internal structure (on magnification) filamentous.

Glycerin Agar Stroke. (2 weeks). Slow, scanty, growth; raised; dry; rough; grayish. Much scantier growth than No. 1 A. Becoming thick, heavily wrinkled and faintly pigmented, light buff (R) after 2 months. Greasy and not easily emulsifiable.

Dextrose Agar Stroke. Similar to growth on Glycerin Agar, but slower and poorer development.

Nutrient Agar Stroke. Very poor growth. Not as good as on Glycerin or Dextrose agar.

Dorset's Egg Medium. (2 weeks). Poor growth. Isolated, raised, slightly moist grayish colonies. Medium not darkened or digested.

Glycerin Broth. (2 weeks - in flasks). Poor growth. Fragile gray pellicle, which climbs part way up the sides of the flask. Liquid clear. Pellicle becomes thicker, and wrinkled, with age.

Loeffler's Blood Serum. (2 weeks). Poor growth. Small, isolated, raised, slightly moist, grayish colonies. No liquefaction or digestion of the medium. Becoming (2 months) pale buff.

Glycerin Potato. (2 weeks). Fairly good growth; slowly spreading; raised; thick and very crumpled; grayish-white, developing (in 2 months) a yellowish pigmentation.

Gelatin Stab. No growth.

III. Biochemical Features.

Litmus and Purple Milk. (2 weeks). No change.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. - pH 6.8.

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Very scanty growth, even on the surface of the medium. No darkening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature (on Glycerin Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth	44°C.
Minimum temperature for growth	35°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.8 - 7.6.

Limits of pH for growth; from 5.0 to 9.5.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

2. Mycobacterium tuberculosis bovis.

I. Morphology. (Studied from B.P.Agar cultures at 37°C.).

Rods, varying in size from 1 to 5.0μ in length by 0.2 to 0.5μ in width, in 24-hour old culture. After this, the size of the organism materially decreases. In 48-hour old cultures, the size of the bacilli vary from 0.5 to 4.0μ in length, with the majority of cells about 2.5μ long by about 0.3μ wide. The rods are straight or slightly curved. Longer, curved, thread-like forms are rarely observed.. No branching. They lie singly, but most often in parallel pairs.. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). The organism does not stain Gram-positive by this technique. The cells appear but indistinctly stained, the larger proportions remaining unstained. (Pl. 3, figs. 1 and 2).

(Hucker). As many cells unstained or staining a faint pink as cells that stain blue. After 14 days growth, the bacilli appear a little more strongly Gram-positive, but this characteristic is very indefinite. (Pl. 3, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). The bacilli stain a solid red - Acid-alcohol-fast. There are a few cells, however, in 24-hour old cultures that do

not take the red stain. The number of these cells very quickly diminishes as the period of incubation increases. (Pl. 3, figs. 5 and 6).

(Gabbet). As with Ziehl-Neelsen's technique. In neither the Gabbet nor the Ziehl-Neelsen techniques do the cells appear unevenly stained. (Pl. 3, figs. 7 and 8).

Muir's Capsule Stain.(3 months on Glycerin Agar). The individual cells show a very delicate enveloping capsule by this stain. Numbers of cells lie grouped together in a mass of gummy material.

II. Cultural Characters. (at 37°C.).

Gelatin colonies. (10 days).

Surface colonies. Very slow, poor development; colonies minute; round; convex; entire; internal structure floccose. No liquefaction.

Dextrose Agar colonies. (7 days).

Surface colonies. Good growth, from 1 to 3.0 m.m. in diameter; irregular; slightly wrinkled; raised; moist; edge uneven and lobate; internal structure filamentous. Becoming (2 weeks) pale ochraceous buff (R).

Deep colonies. Filamentous. Very small (0.25 to 0.5 m.m.).

Glycerin Agar Stroke.(48 hours). Good growth; spreading flat at first, soon becoming thickly raised; moist; heavily wrinkled and heaped up, like the growth of

Mycobacterium tuberculosis hominis (1 A); edge undulate; at first, grayish, soon turning a pale orange yellow and finally becoming Capucine Orange. Becoming stringy and not easily emulsifiable.

Dextrose Agar Stroke. Similar to growth on Glycerin. Growth takes place just as rapidly and luxuriantly on Dextrose as on Glycerin. Pigmentation does not become quite so deep as on Glycerin. **(Pl.27, fig. 1. D).**

Nutrient Agar Stroke. (48 hours). Good growth but slower and not as abundant as on glycerin or Dextrose agar. Moist; spreading; pale yellow; becoming slightly wrinkled.

Dorsett's Egg Medium. (2 weeks). Good growth; slightly spreading; raised; moist; wrinkled; light Ochraceous Buff (R). Medium not darkened or digested.

Glycerin Broth. (3 days-in flasks). Thin, veil-like, grayish pellicle, easily broken into soft, silky flakes, which settle slowly. The liquid is at first clear but soon becomes very turbid from this easily broken and rapidly growing pellicle. The pellicle, however, becomes (2 weeks) much thicker, more wrinkled and pale buff colored. **(Pl. 31, fig. 1. A).**

Loeffler's Blood Serum. (48 hours). Good growth; spreading; raised; slightly moist becoming dry (2 weeks); granularly rough; pale ochraceous buff (R). No digestion or liquefaction of the medium.

Glycerin Potato. (48 hours). Good growth; filiform;

raised; dry, granularly rough; grayish becoming (3 weeks) spreading; thickly raised; crumpled; orange buff (R).

Gelatin Stab. Grayish, raised, surface growth. Scanty, filmy growth in stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Grayish pellicle growth. Becoming alkaline. No coagulation or peptonization.

Carbyhydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks) pH 7.0.

Nitrate Broth and Agar. Strong reduction of nitrates to nitrites in 48 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. No growth in the medium. Reddish brown surface growth. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature (on Dextrose Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth.	52°C.
Minimum temperature for growth.	15°C.

Relation to Reaction of the Medium.

Optimum H-ion conc., about pH 6.8 - 7.3.

Limits of pH for growth; from 4.0 to 10.0.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

3. Mycobacterium paratuberculosis.

I. Morphology. (Studied on Glycerin Agar after 3 months incubation at 37°C.).

Rods, very short and slender, 0.5 to 1.5 μ long by 0.2 to 0. 4μ wide. Majority about 0.9μ long. Straight or very slightly curved; sometimes unevenly swollen. No long forms observed. The cells lie singly but generally in parallel formation. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). The organism stains with difficulty by this method - appearing a very pale almost indistinct blue. (Pl. 4, fig. 1).

(Hucker). As with the Kopeloff and Beerman technique. (Pl. 4, fig. 2).

Acid-Fast Stain. (Ziehl-Neelsen). Distinctly acid-alcohol-fast. The cells stain an even solid red. (Pl. 4, fig. 3).

(Gabbet). As with the Ziehl-Neelsen stain. Acid-fast. (Pl. 4, fig. 4).

Muir's Capsule Stain. No capsules could be demonstrated.

II. Cultural Characters.

Glycerin Agar Stroke. After 3 months incubation, a hardly visible almost transparent growth in the formation of minute colonies was observed.

Glycerin Broth. There was no visible sign of growth after 6 months incubation. No visible growth was obtained on any other media used to cultivate the other organisms of the genus.

4. Mycobacterium avium.

I. Morphology. (Studied from Glycerin Agar at 40°C.).

Rods, very short and slender, varying in size from 0.9 to 2.5 μ long by 0.1 to 0.3 μ wide; very definitely curved. Longer more highly curved rods 7.0 to 9.0 μ long found occasionally occurring in cultures (14 days old), but these forms are very rare. The rods lie singly, and often parallel to each other. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Distinctly Gram-positive, the cells staining an even intense blue. (Pl. 4, fig. 5).

(Hucker). Gram-positive also, but staining less intensely and appearing more slender than by the Kopeloff and Beerman technique. (Pl. 1, fig. 6)

Acid-Fast Stain. (Ziehl-Neelsen). Distinctly acid-alcohol-fast, the rods staining an even red. (Pl. 4, fig. 7).

(Gabbet). As with Ziehl-Neelsen stain. (Pl. 4, fig. 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). The cells stain very unevenly; often distinctly bipolarly. No individual capsules could be observed, but long strands of faintly staining gummy-like material are observed, in which the bacteria held.

II. Cultural Characters. (at 40°C.).

Gelatin colonies. No growth obtained at 20°C.

Dextrose Agar colonies. (2 weeks). Very minute; poor development; irregularly round; raised; smooth; moist; edge uneven; internal structure (on magnification) filamentous.

Glycerin Agar Stroke. (14 days). Slow growth. Isolated colonies, small; raised; convex; moist; glistening; grayish-white; coalescing in time to form a moist, raised, smooth but verrucose, grayish, spreading streak, greasy but easily emulsifiable. (Pl. 29, fig. 2. A).

Dextrose Agar Stroke. Similar to Glycerin Agar Slant.

Nutrient Agar Stroke. Very scanty, poor growth; growing more slowly and not as luxuriantly as on Dextrose or Glycerin.

Dorset's Egg Medium. (2 weeks). Small; isolated; raised; grayish-white; moist colonies, coalescing to form a smooth, moist grayish growth which becomes creamy in color. Medium not darkened or digested.

Glycerin Broth. (grown in flasks). For the first 4 - 7 days, growth takes place slowly at the bottom of the flask. Then suddenly a thin very fragile pellicle starts to develop which increases in thickness with age and becomes wrinkled, climbing up the sides of the flask. The liquid remains clear. Occasionally a pellicle starts to develop slowly from the first.

(Pl. 31, fig. 2. B).

Loeffler's Blood Serum. (14 days). Poor growth. Small, grayish moist colonies, becoming creamy. No digestion or liquefaction of the medium.

Glycerin Potato. (10 days). Slow development. At first small, moist, grayish, raised colonies, becoming (3 weeks) abundant; spreading; raised; slightly moist almost dry; verrucose; very pale creamish gray.

Gelatin Stab. (3 weeks). No growth. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk(1 month). No change.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 7.2

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. No darkening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature (on Glycerin Agar).

Optimum temperature for growth	40°C.
Maximum temperature for growth.	44°C.
Minimum temperature for growth	30°C.

Relation to Reaction of the Medium (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.8 - 7.3

Limits of pH for growth; from 5.0 to 8.5.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

5. Mycobacterium piscium.

I. Morphology. (Studied from B.P. Agar cultures at 25°C.).

Rods, very short and slender, 0.5 to 2.0 μ long by 0.2 μ or less in width. Majority about 0.8 to 1.0 μ long. Straight or slightly curved. No long thread-like, branched, or beaded forms observed. The rods lie singly and in parallel arrangements. There is no morphological change with increase in age. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). The organisms do not stain by Gram. The cells stain a faint pink. (Pl. 5, fig. 1).

(Hucker). The cells stain very faintly pink. Not Gram-positive. They appear more slender by this than by the Kopeloff and Beerman technique. (Pl. 5, fig. 2).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol fast. The cells stain blue. (Pl.5, fig. 3).

(Gabbet). Not acid-fast. (Pl.5, fig. 4).

Muir's Capsule Stain. (1 month on Glycerin Agar).

The individual cells show small indistinct capsules.

II. Cultural Characters. (at 25°C.).

Gelatin colonies. (10 days at 20°C.). Small; about 1 m.m. in diameter; round; convex; smooth; moist and glistening;

entire edge; internal structure finely granular. No liquefaction.

Dextrose Agar colonies. (7 days).

Surface colonies. Small, about 1.0 m.m. in diameter; irregularly round; flat; smooth; moist; radiate; edge lobate; internal structure (on magnification) granular.

Deep colonies. Similar but very minute.

Glycerin Agar Stroke. (48 hours) Moderate growth; spreading; thin; flat; moist; smooth; transparent at first, becoming opaque; pale greenish yellow, becoming Light Chalcedony Yellow (R); slimy.

Dextrose Agar Stroke. Similar to growth on Glycerin. (Pl. 30, fig. 2.C).

Nutrient Agar Stroke. (48 hours). Growth not as rapid or as abundant as on Glycerin or Dextrose Agar. Scanty, moist, glistening, transparent, edge undulate. Pale Glass Green (R).

Dorset's Egg Medium. (2 weeks). Very scanty; spreading; flat; smooth and moist; pale Glass Green (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Thin, reticulately wrinkled pellicle that folds up like a piece of parchment, does not adhere to the sides of the flasks and does not break into flakes and settle to the bottom. Pale green in color. The liquid is at first clear but soon becomes turbid with greenish flocculent sediment.

Loeffler's Blood Serum. (2 days). Very scanty, moist, whitish growth. (3 weeks) Scanty, flat, moist, pale greenish growth. No liquefaction or digestion of medium.

Glycerin Potato. (48 hours). No visible growth. (10 days). Spreading; rough; wrinkled; dry; pinkish buff. (R).

Gelatin Stab. Pale greenish, raised, moist, surface growth. Very scanty, transparent, filmy growth at upper end of stab. No growth at bottom of stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. (7 days). Greenish pellicle. Alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas formed from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 6.2.

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. No growth in the medium. Greenish surface growth. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature (on Dextrose Agar).

Optimum temperature for growth	20°- 25°C.
Maximum temperature for growth	32°C.
Minimum temperature for growth	10°C.

Relation to Reaction of the Medium (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.8.

Limits of pH for growth; from 5.0 to 10.0

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

6. Mycobacterium marinum.

I. Morphology. (Studied from B.P.Agar. Cultures at 25°C.).

Rods, short and slender, 1.5 to 4.0μ long by 0.3 to 0.5 μ wide; occasionally a longer curved rod up to 9.0 μ long is observed, but these are very rare. Majority of cells are about 2.0 μ long by 0.3 to 0.5 μ wide. The rods are curved and are generally of an even thickness throughout their length. There is also rarely observed a short coccoid cell about .5 to 1 μ long, which stains intensely by the Gram method. There is a very marked parallelism in the arrangement of the organisms, all the cells sometimes seeming to lie in parallel pairs or packets. No branched forms were observed. Non-motile. No spores produced.

Staining Reactions.

Gram-Stain.(Kopeloff and Beerman). The organisms do not stain by this technique, neither in young (24-hour) nor in older (14 days) cultures.

(Hucker). Staining very weakly. Only a few cells in a field with numerous organisms stain distinctly blue; the greater majority stain very weakly or a faint pink. The Gram-positivity is doubtful. (Pl. 5, fig. 5 and 6).

Acid-Fast Stain. (Ziehl-Neelsen). Very distinctly acid-alcohol-fast, the cells staining a deep red.

In some cells appear clear unstained spots, very similar in appearance to the unstained spore in a stained vegetative cell. (Pl. 5, fig. 7).

(Gabbet). Distinctly acid-fast. Staining as by Ziehl-Neelsen's technique. The granular nature of the organism is brought out more clearly by these staining procedures. (Pl 5, fig. 8).

Note: Age apparently has little effect on the morphology of the organism. The organisms are a little longer in a 24-hour old culture than in a culture 2 weeks old but the arrangement, general appearance, and staining reactions remain the same.

Muir's Capsule Stain. (2 months on Glycerin Agar). It is very difficult to distinguish any individual capsules. Some gummy material, however, which stains very faintly, seems to hold together masses of bacteria.

II. Cultural Characters. (at 25°C.).

Gelatin colonies. (10 days).

Surface colonies. Small, irregularly round, about 1.0 m.m. in diameter; raised; smooth; moist; edge uneven (on magnification); internal structure filamentous.

Deep colonies. Similar but much smaller.

Dextrose Agar colonies. (7 days).

Surface colonies. Small, about 1 - 2.m.m. in diameter; irregularly round; slightly raised; smooth; moist; edge appearing uneven and filamentous when magnified; Orange Chrome (R); internal structure, filamentous.

Deep colonies. Very small, irregular, yellow.

Glycerin Agar Stroke. (4 days). Scanty growth. Isolated colonies small; yellowish; smooth; moist; raised; coalescing to form a smooth, moist, yellowish streak, which with age becomes spreading, and slightly wrinkled, edge undulate; Orange Chrome (R). Becoming very viscous in old culture. (Pl. 29, fig. 2. D).

Dextrose Agar Stroke. Growth similar to that on Glycerin agar. (Pl. 30, fig. 1. A).

Nutrient Agar Stroke. Similar to Glycerin agar slant but poorer, developing more slowly and not growing as abundantly.

Dorset's Egg Medium. (2 weeks). Good growth; spreading; moist; coarsely granular; Orange Crhome (R). Medium not darkened or digested.

Glycerin Broth. (7 days - in flasks). A thick, viscous, orange growth at bottom of the flask. Turbid. No pellicle formation. In tubes, a fragile orange pellicle is formed. (Pl. 32, fig. 2. C).

Loeffler's Blood Serum. (7 days). Scanty growth; isolated colonies, very minute; smooth; moist; slightly raised. When more numerous forming a filiform; smooth; moist streak; capucine yellow (R). No liquefaction or digestion of the medium.

Glycerin Potato. (2 days). Poor growth; becoming thicker; dry; granular; yellow ochre (R).

Gelatin Stab. Orange, filiform growth along line of inoculation. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. (7 days). A very slight acidity developed. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 7.0

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Blackened, filiform, streak in and on the medium. H_2S produced.

IV. Physiology.

Aerobic

Relation to Temperature (on Dextrose Agar).

Optimum temperature for growth	20°- 25°C.
Maximum temperature for growth	37°C.
Minimum temperature for growth	10°C.

Relation to Reaction of the Medium (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.8

Limits of pH for growth; from 5.8 to 10.8.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

7. Mycobacterium ranae.

I. Morphology. (Studied from B.P.Agar cultures at 37°C.).

Rods; 2.0 to 8.0 μ long by 0.3 to 0.5 μ wide in 24-hour old cultures, slender and slightly curved, lying singly and in parallel arrangements. The size of the organisms decreases with age; in a culture, 2 weeks old, the greater number of rods are about 1.0 to 1. 5 μ long. Beaded and barred forms present. Some cells swollen at one or both ends. No branched forms were observed. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). In cultures up to 3 days old, the cells stain evenly and strongly Gram-positive. After this, the cells stain unevenly, having a distinctly beaded appearance. (Pl. 6, figs. 1 & 2).

(Hucker). Weakly Gram-positive. (Pl. 6, figs. 3 & 4).

Acid-Fast Stain. (Ziehl-Neelsen). The organisms are very feebly acid-alcohol-fast in cultures, 24 hours old. In 2 weeks, however, the cells become strongly acid-alcohol-fast. Many beaded forms are also present in older cultures. (Pl. 6, figs. 5 and 6).

(Gabbet). Weakly acid-fast in young cultures. In older cultures (2 weeks) more strongly acid-fast. Blue granules are not uncommon. (Pl. 6, figs. 7 and 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). The cells show a delicate surrounding capsule. Clumps

of bacilli lie together in a mass of gummy-like material.

II. Cultural Characters. (at 37°C.)

Gelatin colonies. (10 days). Very small, about 0.5 to 1 m.m. in diameter; irregularly round, raised; moist; glistening; smooth; internal structure (magnified) floccose. No liquefaction.

Dextrose Agar colonies. (7 days).

Surface colonies. Good growth from 1.0 to 3.0 m.m. in diameter; irregular; raised; surface coarsely granular and segmented; slightly moist; uneven edge; when magnified, filamentous border; internal structure, filamentous.

Deep colonies. Small, slightly creamish yellow. Irregular.

Glycerin Agar Stroke. (48 hours). Good growth, becoming thick; spreading; raised; moist at first, becoming dry; wrinkled; cream-buff (R); pigmentation deeper in the central portion of the growth. Becoming (4 weeks) greasy, somewhat stringy and not easily emulsifiable.

Dextrose Agar Stroke. Growth similar and as abundant as on Glycerin Agar. (Pl. 27, fig. 2. B).

Nutrient Agar Stroke. (48 hours). Moderate, becoming in 3 days thick; spreading; raised; smooth; moist; grayish-white; putrid odor; butyrous.

Dorset's Egg Medium. (2 weeks). Good growth; spreading; raised; moist; glistening; wrinkled like Mycobacterium tuberculosis bovis; antimony yellow (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Grayish flaky pellicle breaking up into flakes and settling to the bottom of the flask. Slight putrefactive odor. (Pl. 31, fig. 1 D).

Loeffler's Blood Serum. (2 days). Good growth; spreading; raised; grayish. At first very moist, smoothly raised, isolated colonies growing into a confluent slant. (7 days). Moist; raised; coarsely granular; warm buff (R). No liquefaction or digestion of the medium.

Glycerin Potato. (48 hours). Very scanty; grayish growth, becoming (2 weeks) very raised, with roughened warty surface; dry; apricot buff (R).

Gelatin Stab. Whitish filiform growth best at surface, very poor in the medium. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Slightly alkaline in 48 hours, becoming strongly alkaline (7 days). Very slight peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 7.0.

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Creamy white surface growth, slowly becoming brownish. No growth in the medium. No blackening or discoloration of the agar.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth.	52°C.
Minimum temperature for growth	10°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.6 - 7.3.

Limits of pH for growth; from 4.0 to 10.0.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

8. Mycobacterium thamnopheos.

I. Morphology. (Studied from B.P. Agar cultures at 25°C.).

Rods, ranging in size from 1.0 μ to 5.0 μ long by about 0.5 μ wide. Occasional rods 7 and 8.0 μ long are found, but the majority of bacilli are about 3 μ long by about 0.3 - 0.5 μ wide. There are also present in cultures, 24 hours old, spherical coccoid cells, 1.0 to 1.5 μ in diameter. The rods are straight or curved, sometimes distinctly swollen in the middle or at the poles. They lie singly or in a parallel arrangement. No branched forms were observed. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Distinctly Gram-positive, staining more intensely by this procedure than by the Hucker modification. Some cells stain bipolarly. (Pl.7, figs. 1 and 2).

(Hucker). Gram-positive in cultures, 24 hours and 1 month old. (Pl. 7, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). Non-acid-alcohol fast. In older cultures (1 week and older) the bacilli stain very unevenly: they are distinctly beaded, little bluish granules staining in a very pale pink undistinct cell and giving the appearance very often of a streptococcus. (Pl.7, figs. 5 & 6).

(Gabbet). In young cultures (1 to 4 days old), among the acid-fast bacilli appear a number of cells staining blue, non-acid-fast. In older cultures (6 days) the bacilli stain more evenly and distinctly acid-fast. (Pl.7, figs. 7 & 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). The cells stain very unevenly, sometimes distinctly bipolarly. No distinct capsular material could be demonstrated.

II. Cultural Characters. (at 25°C.).

Gelatin colonies. (10 days).

Surface colonies. Smaller colonies, round, about 1.0 m.m. in diameter; capitate and matte: larger colonies, very irregular, about 3.0 m.m. in diameter; raised and squamose; edge lacerate; internal structure, "curled". No liquefaction.

Deep colonies. Much smaller, round, about 0.5 m.m. in diameter.

Dextrose Agar colonies. (7 days).

Surface colonies. Small about 0.5 to 1.0 m.m. in diameter; irregular; raised and rough; moist; glistening; edge erose; internal structure (magnified) curled.

Deep colonies. Irregular. Lens-shaped or elliptical.

Glycerin Agar Stroke. (48 hours). Good growth; spreading; raised; dry; warty; pale pinkish buff (R); easily emulsified.

Dextrose Agar Stroke. (48 hours). As on glycerin agar. Slightly wrinkled at the bottom of the stroke. (Pl. 29, fig. 1. A).

Nutrient Agar Stroke. (48 hours). Good growth; spreading; raised; dry; surface roughened by smooth warty promincences; white and opaque. In 2 weeks, becoming moist; glistening; pale pinkish buff (R).

Dorset's Egg Medium. (2 weeks). Good growth; spreading; raised; moist in the thicker portions of the growth, dry at the top of the slant; verrucose; pale ochraceous buff (R). Medium not darkened or digested.

Glycerin Broth. (3 days - in flasks). Firm, shell pink, deeply wrinkled pellicle. Clear, but small flocculi break away from the surface growth and settle to the bottom slowly, causing a slight turbidity.(Pl.32,fig.1. D).

Loeffler's Blood Serum. (48 hours). Poor growth. (7 days). Isolated colonies; very small; raised; convex; dry. When more numerous, they coalesce to form a dry verrucose, ligh buff (R), growth. No digesti on or liquefaction of the medium.

Glycerin Potato. (4 days). Moderate growth; raised almost hemispherical colonies; dry; light buff. Becoming (2 weeks) slightly spreading; surface finely granular; edge undulate. After 1 month, turning a tawny brown.

Gelatin Stab. (7 days). Good surface growth; grayish-white; raised; rough; slightly moist. Very scanty, hardly visible growth along line of inoculation. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. (1 week). Unchanged.

In 2 weeks, becoming alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 7.0.

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Dark brown, almost black surface growth. No growth in the medium and no discoloration.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar):

Optimum temperature for growth	25°C.
Maximum temperature for growth	35°C.
Minimum temperature for growth	10°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 7.3 to 8.0

Limits of pH for growth; from 5.0 to 11.0

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

9. Mycobacterium chelonei.

I. Morphology. (Studied from B.P. Agar cultures at 25°C.).

Small, slender rods, 1.5 to 5.0 μ long by 0.2 to 0.3 μ wide; with the occasional longer and thicker form. Slightly curved, some almost comma-shaped. With age, the bacilli become a little shorter; in cultures, 2 weeks old, the length of the majority of organisms is about 1.5 to 2.0 μ. They occur singly, in short chains of two or three cells, and also in parallel pairs. Beaded forms are very common. No branched forms were observed. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Gram-positive. A few bacilli stain distinctly and evenly, but the majority of cells stain very unevenly, being distinctly beaded; these small blue granules or beaded portions appear distinct and separate, resembling a chain of very small streptococci; the vegetative cell is hardly distinguishable. (Pl. 8, fig. 1).

(Hucker). Young cultures (1 and 2 days old) stain distinctly Gram-positive, with a number of cells showing a beaded structure. Later, the bacilli lose this beaded appearance and become more weakly Gram-positive, a large number staining distinctly Gram-negative. (Pl. 8, fig. 2).

Acid-Fast Stain. (Ziehl-Neelsen). Acid-alcohol-fast.

In older cultures (2 weeks) there also appear a few blue (not-acid-alcohol-fast) granules in the red cells. (Pl. 8, fig. 3).

(Gabbet). Acid-fast. The cells stain evenly. (Pl. 8, fig. 4).

Muir's Capsule Stain. (2 months on Glycerin Agar) Long strands of gummy, capsular material are observed in which the bacilli lie massed together.

II. Cultural Characters. (at 25°C.).

Gelatin colonies. (10 days).

Surface colonies. About 1 to 3 m.m. in diameter; irregularly round; raised; flatter at the edges; surface, moist and glistening; matte; edge lobed; internal structure granular. With a low magnification, the colony resembles a piece of marble smoothly cut into folds and rounds. No liquefaction.

Deep colonies. Very small, round, creamy in color.

Dextrose Agar colonies. (7 days).

Surface colonies. About 0.5 to 1 m.m. in diameter; irregularly round; flat with roughened surface; dry; edge lobate; under the microscope, the colony closely resembles the "anthrax" colony - curled.

Deep colonies. Very irregular, small, dense.

Glycerin Agar Stroke. (48 hours). Good growth; thick; spreading; raised; moist; a very characteristic roughness, like "plush"; pale olive-gray (R).

Dextrose Agar Stroke. Similar but more abundant growth than on Glycerin Agar. (Pl. 28, fig. 2. C).

Nutrient Agar Stroke. (24 hours). Very scanty. (48 hours). Moderate. (4 days). Good growth; spreading; raised; moist; at first smooth, becoming slightly roughened (plush-like); white; butyrous. Medium becoming slightly darkened after some days.

Dorset's Egg Medium. (2 weeks). Good growth; spreading; raised; slightly moist; smooth and verrucose; cartridge buff (R). Medium not darkened or digested.

Glycerin Broth. (3 days - flasks). Clear with thick, grayish-white, wrinkled, yeast-like pellicle. When slightly shaken a membranous mass from the pellicle settles to the bottom of the tube, causing a thick sediment. The liquid remains clear. (Pl. 32, fig. 2.D).

Loeffler's Blood Serum. (48 hours). Scanty growth. Isolated colonies, raised; dry; crumb -like, grayish-white; coalescing to form atypical; spreading; dry; plush-like; grayish growth. No liquefaction or digestion of the medium.

Glycerin Potato. (48 hours). Thick; wrinkled; gray.

Gelatin Stab. Whitish surface growth; scanty growth along stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. In 10 days, developing a slight alkalinity. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). Ph 7.0

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brownish-gray surface growth. No growth in the medium. No discoloration of the agar.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	25°C.
Maximum temperature for growth	32°C.
Minimum temperature for growth	18°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.8 - 7.1

Limits of pH for growth; from 5.0 to 10.8

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

10. Mycobacterium smegmatis.

I. Morphology. (Studied from B.P.Agar cultures at 37°C.).

Rods, slender, straight or slightly curved, 1.5 to 4 μ long by 0.3 to 0.4 μ wide, in cultures 24 hours old. In older cultures (after 2 days) the bacilli get very much smaller; short thicker forms, about 1 μ long 0.5 to .75 μ wide, resembling somewhat oval cocci, appear. The rods lie irregularly, in short chains, and in parallel arrangement. In the young culture there are also longer thread-like forms occasionally observed. No branched forms were observed. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). In young (24 hour) cultures, the rods appear very granulated. The vegetative cell is very indistinctly stained, a pale pink, but it is dotted with a number of distinctly Gram-positive granules. In older cultures, the vegetative cell becomes even more indistinct, only the granules being stained, appearing like cocci, grouped in pairs and short chains. (Pl.9, figs. 1 and 2).

(Hucker). Staining weakly by Gram in young (24 hour) cultures, a number of cells

being distinctly beaded. In older cultures (3 days and older), the preparation consists almost entirely of very short, thick coccoid rods, which stain deeply by Gram.(Pl.9, figs. 3 & 4).

Acid-Fast Stain. (Ziehl-Neelsen). In young culture (24 hours), some cells stain distinctly red, some are beaded with darkly staining granules, and some stain a pale blue. Soon, however, the culture consists almost entirely of small coccoid-like cells, which are strongly acid-alcohol-fast. (Pl. 9, figs. 5 and 6).

(Gabbet). In young (24 hours) cultures, the majority of cells are acid-fast, staining solidly. There are, however, some cells with a beaded appearance, in which dark granules are seen. In older cultures, the short coccoid cells stain strongly acid-fast. (Pl. 9, figs. 7 and 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). Like Mycobacterium stercusis; no distinct individual capsules were observed, but there were clumps of bacteria held together in a mass of gummy-like material.

II. Cultural Characters. (at 37°C.).

Gelatin colonies. (10 days).

Surface colonies. About 1.5 m.m. in diameter; irregularly round; raised; squamose; edge lacerate; internal structure (on magnification) floccose. No liquefaction.

Deep colonies. Much smaller, round, entire, about 0.3 m.m. in diameter.

Dextrose Agar colonies. (7 days).

Surface colonies. Small; about 1.5 - 2.0 m.m. in diameter; irregularly round; raised; moist and glistening; smooth but uneven; edge erose on magnification; grayish-white turning cream; internal structure filamentous.

Deep colonies. Very irregularly shaped, smaller and denser.

Glycerin Agar Stroke. (48 hours). Abundant; spreading; thick; moist; smooth, becoming wrinkled by long folds; gray, becoming light buff (R). With age becomes dry; butyrous.

Dextrose Agar Stroke. Similar to growth on Glycerin Agar Stroke. Becoming, in 2 weeks, orange buff (R). (Pl. 27, fig. 2. C).

Nutrient Agar Stroke. Growth not as rapid or abundant as on Dextrose or Glycerin. Pigmentation is not as deep.

Dorset's Egg Medium. (2 weeks). Good growth; spreading; raised; moist; surface slightly roughened by

a few warty prominences; Capucine Yellow (R). Medium not darkened or digested.

Glycerin Broth. (3 days - in flasks). Thin, flaky, dry, grayish pellicle. Liquid clear with flaky sediment. Disagreeable sour odour. (Pl. 31, fig. 1. C).

Loeffler's Blood Serum. (48 hours). Thick; slightly spreading; moist; smooth; lumpy; becoming slightly wrinkled (7days); ochraceous buff (R). No digestion or liquefaction of the medium.

Glycerin Potato. (48 hours). Dull, dry, raised, rapid growth.

Gelatin Stab. (7 days). Scanty whitish surface growth and along line of inoculation. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. In 5 days developing an alkalinity. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks) pH. 6.8.

Nitrate Broth and Agar. Nitrates reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brownish surface growth. No growth in the medium. No blackening of the medium.

IV. Physiology.

Aerobic

Relation to Temperature.(on Dextrose Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth	52°C.
Minimum temperature for growth	15°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.6 - 7.8

Limits of pH for growth; from 4.4 to 10.8.

Indol Agar. Indigotin not formed

Blood Agar Plate. No haemolysis.

11. Mycobacterium butyricum.

I. Morphology. (Studied from B.P. Agar cultures at 35°C.).

Very short rods, coccoid almost in appearance, about 1 μ in length by about 0.5 to 1.0 μ in width. Generally wider in the middle and rounding off at the edges. No branching or thread-like forms were observed. The culture has the same appearance at the end of seven weeks that it has in 24 hours. The staining reactions likewise, remain the same in old and young cultures. The bacilli lie singly, in pairs, and in irregular masses. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Very feebly Gram-positive, the cells staining mauve. (Pl. 10, fig. 1).

(Hucker). Gram-positive, staining more distinctly than by the Kopeloff and Beerman technique. (Pl.10, fig. 2).

Acid-Fast Stain. (Ziehl-Neelsen). Distinctly acid-alcohol fast; the bacilli stain evenly. (Pl.10, fig. 3).

(Gabbet). Acid-fast, the same as with the Ziehl-Neelsen technique. (Pl.10, fig. 4).

Muir's Capsule Stain.(2 months on Glycerin Agar).

A distinct delicate capsule surrounding the individual cell may be observed. The cells also lie grouped together in a mass of gummy material

II. Cultural Characters. (at 35°C.).

Gelatin colonies. (10 days).

Surface colonies. Minute; almost pin-point colonies; round; moist; glistening; smooth; pale cream; internal structure coarsely granular. No liquefaction.

Deep colonies. Similar.

Dextrose Agar colonies. (7 days).

Surface colonies. Very small; about 0.5 to 1.0 m.m. in diameter; round; convex; smooth; moist; glistening; entire; grayish-white turning Capucine Yellow (R); internal structure, granular.

Deep colonies. Lens-shaped. Vary small. Yellow.

Glycerin Agar Stroke. (48 hours). Good growth; spreading; moist and shiny; smooth. In 10 days, although remaining smooth, the edge becomes slightly lobate and the surface shows a terraced border; Capucine Yellow (R). Becoming stringy in older cultures.

Dextrose Agar Stroke. (48 hours). Good growth; slightly spreading; moist; glistening; smooth

becoming slightly wrinkled in 7 days; Capucine Yellow. Stringy in old cultures.

Nutrient Agar Stroke. (Pl. 27, fig. 2. A). Poor, restricted growth. Smooth; moist; glistening; Capucine Yellow (R).

Dorset's Egg Medium. (2 weeks). Filiform streak; raised almost hemispherical; smooth; moist; pale orange yellow (R). Medium not darkened or digested. Isolated colonies, small, semi-circularly raised; round; moist; smooth.

Glycerin Broth. (3 days - in flasks). Wrinkled pellicle; becoming thicker; adherent to the sides of the flask; yellowish. Medium turbid, with pale yellowish sediment. (Pl. 32, fig. 2. A).

Loeffler's Blood Serum. (48 hours). Moderate growth. Isolated colonies, very small, round, raised, smooth, moist. Where more numerous they form a smooth, raised, yellowish streak, becoming (7 days) warm buff (R). No digestion or liquefaction of the medium.

Glycerin Potato. (48 hours). No growth. (10 days). Spreading; raised; moist; glistening; rough; Light Cadmium (R).

Gelatin Stab. (7 days). Small, raised, smooth, yellow surface growth. Yellowish filiform growth in the medium. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Developing a slight alkalinity. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 7.0.

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Yellowish growth at surface and along line of inoculation. No darkening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	35°C.
Maximum temperature for growth	44°C.
Minimum temperature for growth.	15°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.6 - 7.1

Limits of pH for growth; from 5.5 to 10.5

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

12. Mycobacterium berolinensis.

I. Morphology. (Studied from B.P.Agar cultures at 37°C.).

Rods, ranging in size from 1.0 to 3.5 μ in length by 0.5 μ or less in width; majority about 2.0 μ long. The organisms get slightly smaller with age. The bacilli are straight or slightly curved. Some cells are swollen in the middle, others at the pole or poles. A few longer filamentous forms sometimes present in young and old cultures. The rods lie singly and very often in parallel arrangement. No branched forms were observed. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain.(Kopeloff and Beerman). The majority of bacilli stain very weakly Gram-positive; a few stain deeply, but many stain feebly Gram-negative. (Pl. 10, fig. 5).

(Hucker). Gram-positive. A "beaded" staining is very common. (Pl. 10, fig. 6.).

Acid-Fast Stain.(Ziehl-Neelsen). Acid-alcohol-fast. The bacilli stain evenly. (Pl. 10, fig. 7).

(Gabbet). Acid-fast, the cells staining evenly. (Pl. 10, fig. 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). A delicate, sometimes wider capsule observed around an individual cell. Groups of bacteria lie. often, in a mass of gummy-like material.

II. Cultural Characters. (at 37°C.).

Gelatin colonies.(10 days).

Surface colonies. Small; about 1.0 m.m. in diameter; irregularly round; convex; moist; surface deeply segmented; edge lobulate; pale cream; internal structure floccose.

Deep colonies. Very small, about 0.5 m.m. in diameter, round.

Dextrose Agar colonies.(7 days).

Surface colonies. Small, about 1.0 to 1.5 m.m. in diameter; irregularly round; convex; moist; glistening; yellow; internal structure, filamentous.

Deep colonies. Small, yellow, lens-shaped.

Glycerin Agar Stroke. (48 hours). Abundant growth; spreading; raised; at first moist, becoming dry; thick; very slightly wrinkled; pinkish buff.

Dextrose Agar Stroke. (48 hours). Abundant; spreading; raised; moist; thick; wrinkled; creamy.(Pl.28,fig.1.D).

Nutrient Agar Stroke. (48 hours). Good growth; sprading; moist at the edges but dry in the deeper portions of the growth; slightly wrinkled; grayish-white, becoming (2 weeks) pale pinkish, "thicker", more moist and hard and stringy.

Dorset's Egg Medium.(2 weeks). Good growth; filiform to slightly spreading; slightly raised; dry; gramular; Capucine Orange (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Thick, firm, grayish, wrinkled pellicle adhering to the sides of the flask. Very slight turbidity caused by small flocculi settling to the bottom of the flask. Disagreeable sour odour. (Pl. 31, fig. 1. B).

Loeffler's Blood Serum. (48 hours). Good growth; filiform; raised; slightly moist; roughened surface; Maize Yellow (R). No digestion or liquefaction of the medium (2 weeks).

Glycering Potato. (48 hours). Good growth. Slightly moist turning dry; raised; wrinkled; chamois colored.

Gelatin Stab. (7 days). Scanty, creamish, raised, rough surface growth. Scanty filiform growth along stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Creamy pellicle. Alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks) pH 6.4

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Black surface growth. No growth in the agar. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth	52°C.
Minimum temperature for growth	20°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 7.1 - 7.6

Limits of pH for growth; from 4.4 to 10.5.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

13. Mycobacterium stercusis.

I. Morphology. (Studied on B.P. Agar cultures at 37°C.). (24 hours old). Very small slender rods, 1.5 to 4.0 μ long by 0.25 to 0.5 μ wide; majority 3.0 μ to 0.4 μ. A few longer thread-like forms occur. The cells lie singly, more often in short chains and in parallel arrangement. In older cultures (3 days and older), the size of the bacillus is somewhat reduced, some cells appearing almost coccus-like. No branched forms were observed. Non-motile. No spores produced.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Weakly Gram-positive. A few cells stain strongly, the greater number stain very unevenly, deep blue granules appearing in faintly pink cells. (Pl. 11, figs. 1 and 2).

(Hucker). More strongly Gram-positive. The cells stain evenly, only very few showing a granular appearance. (Pl. 11, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). Acid-alcohol-fast in old cultures. In young (24 hour) cultures some cells stain a pale blue. (Pl. 11, figs. 5 and 6).

(Gabbet). Acid-fast. In this

technique, as with the Ziehl-Neelsen, sometimes deeply staining granules appear in the rod. (Pl. 11, figs. 7 and 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). No distinct capsule surrounding the individual cell could be distinguished, but clumps of bacteria lie grouped together in a mass of very faintly staining gummy-like material.

II. Cultural Features. (at 37°C.).

Gelatin colonies. (10 days).

Surface colonies. Very slow development. Colonies minute; punctiform; white; internal structure (under magnification) floccose. No liquefaction.

Deep colonies. Pin-point colonies.

Dextrose Agar colonies. (7 days).

Surface colonies. Irregularly round; 1 to 2 m.m. in diameter; raised; moist; smooth; grayish-white. Under a low magnification the filamentous nature of the growth and the uneven edge can be distinguished.

Deep colonies. Irregular; lens-shaped.

Glycerin Agar Stroke. (48 hours). Abundant; spreading; thick; moist; smooth, becoming folded; grayish-white. After 2 weeks, growth becomes Sea-Foam Yellow. (R).

Dextrose Agar Stroke. Spreading; raised; thick; moist; surface segmented; grayish-white at first, turning reddish. (Pl. 27, fig. 1. B).

Nutrient Agar Stroke. (48 hours). Abundant; spreading; thick; moist; glistening; smooth; gray; butyrous. Medium unchanged.

Dorset's Egg Medium. (2 weeks). Good growth; filiform; raised; moist; smooth; ochraceous buff (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Slight turbidity. Thin, slightly greenish membranous pellicle that is very easily broken up and settles to the bottom of the flask in large flakes. (Pl. 31, fig. 2. D).

Loeffler's Blood Serum. (48 hours). Good growth; spreading; moist; smooth; a very pale creamy yellow; turning (7 days) Cream Color (R). No digestion or liquefaction of the medium.

Glycerin Potato. (48 hours). Slight growth; flat; pale yellow; dull; dry; (10 days) Buff Yellow (R).

Gelatin Stab. Filiform growth; best at top; creamy; moist; raised surface growth. Poor growth along stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Becoming alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 6.4.

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. A scanty, whitish growth along stab. No blackening of the medium.

IV. Physiology.

Aerobic

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth	52°C.
Minimum temperature for growth	15°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.6 - 7.3

Limits of pH for growth; from 5.0 to 10.5.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

14. Mycobacterium phlei.

I. Morphology. (Studied from B.P. Agar cultures at 37°C.).

Small, slender rods, 1.5 to 3.5 μ long by 0,2 to 0.5 μ wide in cultures, 24 hours old; majority 1.5 μ by 0.3 μ. The bacilli are straight or slightly curved, sometimes appearing comma-shaped. Beaded forms are common. In young cultures, there is occassionally a longer highly curved rod 6.0 to 7.0 μ long. The bacilli lie singly, in clumps and frequently show a parallel arrangement. No branched forms were observed. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). The bacilli are very weakly Gram-positive, staining mostly as small blue granules in a pale indistinctly stained vegetative cell. A few cells stain evenly but the great majority show a granular staining. (Pl. 12, figs. 1 & 2).

(Hucker). Weakly Gram-positive, but staining evenly. After 8 days, the bacilli stain more weakly. (Pl. 12, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). Acid-alcohol-fast. Some cells stain evenly red, some very unevenly (beaded with bluish granules) and some do not stain red at all. The number of the acid-alcohol-fast bacilli increases with the age of the culture. (Pl. 12, figs. 5 and 6).

(Gabbet). Acid-fast, staining similarly to the preparations, stained by the Ziehl-Neelsen technique. (Pl. 12, figs. 7 and 8).

Muir's Capsule Stain.(3 months on Glycerin Agar). No distinct individual capsules could be distinguished, but numbers of bacilli lie grouped together in a mass of capsular material.

II. Cultural Characters. (at 37°C.).

Gelatin colonies (10 days).

Surface colonies. Very small, 0.5 to 1 m.m. in diameter; irregularly shaped; raised; moist; glistening; finely granular; edge uneven; internal structure (magnified) floccose. No liquefaction.

Deep colonies. Very small o.3 m.m. or less in diameter. Round .

Dextrose Agar colonies. (7 days). Small about 1 m.m. in diameter; irregular; raised; moist; slightly roughened surface; delicately radiately ridged; edge filamentous; pale yellow; internal structure, filamentous.

Glycerin Agar Stroke. (48 hours). Good growth; spreading; raised; dry with a furry appearance; roughened surface (not wrinkled); yellow turning to orange (R).

Dextrose Agar Stroke. Growth similar to Glycerin Agar Stroke culture. Growth is just as rapid and abundant on this medium as on Glycerin Agar.

Nutrient Agar Stroke. (48 hours). Good growth; (Pl. 27, fig. 2. D). spreading; raised; dry; roughened surface; at first white turning pale yellow.

Dorset's Egg Medium.(2weeks). Good growth; spreading; raised; dry; granular; Mikado Orange (R).

Glycerin Broth. (3 days in flasks). Thin, transparent, slightly pinkish, adherent pellicle, slightly wrinkled in long folds. Clear with flaky sediment which settled as it broke away from the pellicle. In 7 days, the pellicle became orange colored, more wrinkled, climbed part way up the sides of the flask, and was very adherent.(Pl. 33,fig. 1. B).

Loeffler's Blood Serum. (48 hours). Abundant; spreading; raised; dry; granular; cream. (1 week). Spreading;dry; rough; Buff Yellow (R). No liquefaction or digestion of the medium.

Glycerin Potato.(48 hours). Rapid growth; thick; dry; rough; Cadmium orange (R).

Gelatin Stab. Orange, filiform, opaque growth; thick, raised, surface growth. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Becoming slightly alkaline in 7 days. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 6.8.

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Dark brown, almost black, surface growth. No growth in the medium. No blackening or discoloration of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature. (On Dextrose Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth	58°C.
Minimum temperature for growth	20°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.8 - 7 3.

Limits of pH for growth; from 5.5 to 10.5.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

15. Mycobacterium graminis.

I. Morphology. (Studied from B.P. Agar cultures at 37°C.).

Rods, long and slender, 2.0 to 6.0 μ long by 0.2 to 0.4 μ wide in cultures, 24 hours old. With age, the bacilli become more slender and smaller, 1.5 to 2.0 μ long by about 0.2 μ wide (8 days). The rods are straight or slightly curved. The longer, more thread-like form, up to 10 μ long is occasionally seen in young and older cultures. Beaded and swollen forms are not uncommon. The bacilli are generally arranged in parallel chains. No branched forms were observed. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Weakly Gram-positive. Very uneven staining. The bacilli stain as blue granules in a pale pink vegetative cell. (Pl. 13, figs. 1 and 2).

(Hucker). Gram-positive, with many cells staining very weakly. Granular forms are not commonly observed, by this procedure. (Pl. 13, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). In young cultues, 1 - 5 days old, the bacilli are weakly acid-alcohol-fast, there being many cells which do not show this property. In older cultures (7 days), the bacilli stain more strongly acid-alcohol-fast.

(Pl. 13, figs. 5 and 6).

(Gabbet). The bacilli stain evenly, and are acid-fast in young cultures. In older cultures, they stain more intensely. (Pl. 13, figs. 7 and 8).

Muir's Capsule Stain. (3 months in Glycerin Agar). No distinct capsule surrounding the individual cell could be distinguished, but masses of gummy-like material held numerous bacilli clumped together.

II. Cultural Characters. (at 37°C.).

Gelatin colonies. (10 days.).

Surface colonies. 1. to 2 m.m. in diameter; irregularly round; slightly raised; glistening; coarsely granular; edge deeply lobate and burred; cream-colored; internal structure (magnified) floccose.

Deep colonies. Very small, 0.5 m.m. in diameter; round.

Dextrose Agar colonies (7 days).

Surface colonies. About 2.0 to 6.0 m.m. in diameter; irregular; raised; dry; granular; edge erose and filamentous, "burr-like"; very pale yellow; internal structure, filamentous.

Deep colonies. Smaller, about 1.0 m.m. in diameter, irregularly round. On magnification, burr-like.

Glycerin Agar Stroke. (48 hours). Good growth; spreading; thin; dry; membranous; grayish white. In 7 days becoming thick; spreading; raised; dry; wrinkled by

a mass of thickly woven folds; pale orange yellow (R); adherent growth, not easily emulsifiable.

Dextrose Agar Stroke. Thinner; more membranous type of growth, less heavily wrinkled, deeper yellow-buff yellow (R) than on Glycerin Agar. (Pl.28, fig. 1.B & fig. 2. B).

Nutrient Agar Stroke. Thin; delicate; spreading; slightly moist; with slightly raised and very finely wrinkled surface; grayish white, turning a pale yellowish color.

Dorset's Egg Medium. (2 weeks). Good growth; spreading; raised; slightly moist; finely wrinkled; orange buff (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Strong, firm, grayish pellicle, adherent to and climbing up the the sides of the flask, and not easily broken up. Wrinkled in large folds. Liquid clear. No sediment. With age, the pellicle gets thicker and yellowish. (Pl. 32, fig. 1. B).

Loeffler's Blood Serum. (2 days). Good growth; spreading; raised; thick; dry; rough; grayish-white. (7 days) Light Ochraceous Buff (R). No digestion or liquefaction of the medium.

Glycerin Potato. (2 days). No visible growth. (10 days). Abundant; spreading; thick; dry; wrinkled; pale orange-yellow.

Gelatin Stab. Grayish surface growth and filiform growth in stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Developing a thick, rough, grayish pellicle. Alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 7.0.

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brown surface growth. No growth in the medium. No blackening or discoloration of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth	52°C.
Minimum temperature for growth	18°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 6.8 - 8.5.

Limits of pH for growth; from 4.0 to 10.5.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

16. Mycobacterium actinomorphum.

I. Morphology. (Studied from B.P. Agar cultures (25°C.).

Rods, varying in size from 1.5 to 12.0 μ long by 0.5 to 0.8 μ wide. The longer rods, 6.0 to 12.0 μ long, are generally curved and may show branching, sometimes forming two or three branches. The long rods are more numerous in young culture, although at the end of 7 weeks there are still many long branching forms. The short rods, which become more numerous with the age of the culture, lie grouped together in pairs or short chains. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). In young cultures (24 hours) all the organisms stain intensely by Gram. In older cultures (1 week), although there are many cells staining this intense blue, there are also many cells which stain weakly and the number of these weakly staining cells increases with age. (Pl. 14, figs. 1 & 2).

(Hucker). Gram-positive. The short rods stain distinctly blue, but a large number of the larger rods stain very feebly. The organisms appear more slender by this technique than by that of Kopeloff and Beerman.
(Pl. 14, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol-fast. The bacilli, both the long and short forms, stain blue. (Pl. 14, figs. 5 and 6).

(Gabbet). In young cultures (24 hours), all the bacilli are non-acid-fast. In cultures, one week old and older, the occasional acid-fast short rod is observed. (Pl. 14, figs. 7 & 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). The cells stain unevenly, beaded and bipolarly. No distinct capsules were observed.

II. Cultural Characters. (at 25°C.).

Gelatin colonies. (10 days).

Surface colonies. About 1.0 m.m. in diameter; round; pulvinate; matte; edge entire, when magnified, filamentous; very pale buff; internal structure, floccose. No liquefaction.

Deep colonies. Similar but much smaller.

Dextrose Agar colonies. (7 days).

Surface colonies. About 1.0 to 3.0 m.m. in diameter; round; raised; dry; concentrically ringed with raised centre; arborescent projections from the edge; internal structure, filamentous. The smaller colonies are smooth, white, moist and glistening.

Deep colonies. Round, creamy yellow arborescent burrs.

Glycerin Agar Stroke. (48 hours). Good growth; spreading; raised; dull; dry; surface becoming very wrinkled; edge distinctly lobate; pale sea-shell pink (R) becoming orange pink (R) with a very pale grayish border (10 days); powdery and easily emulsifiable.

Dextrose Agar Stroke. As on Glycerin Agar. (Pl.27, fig.1.C).

Nutrient Agar Stroke. Growth not as abundant as on Dextrose or Glycerin agar. Slightly spreading; dull and dry; a little raised with a few wrinkles; edge undulate; white.

Dorset's Egg Medium. (2 weeks). Good growth. Isolated colonies are small, dry, hemispherical and smooth. When more numerous they coalesce to form a dry, bullate, salmon-buff (R) streak. The medium is not darkened or digested.

Glycerin Broth. (3 days in flasks). Thin, pale pink, opaque, dry pellicle. Pellicle unevenly crossed by long smooth folds (contoured); it is easily broken and the large flakes settle through the clear medium to the bottom of the flask. In two weeks the pellicle climbs up the sides of the flask, becomes more definitely pink, and small strands or threads are suspended from it into the slightly cloudy liquid. (Pl. 33, fig. 2. A).

Loeffler's Blood Serum. (48 hours). Good growth; smooth; moist; raised with warty prominences or

verrucose surface; pale pink. (2 weeks) Dry; pale ochraceous salmon (R). No liquefaction or digestion of the medium.

Glycerin Potato. (48 hours). Dry; crumpled; raised; pale pink becoming orange pink (R).

Gelatin Stab. Filiform growth along line of inoculation. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Becoming alkaline in 7 days. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 6.0.

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brown surface growth. No growth in the butt. Medium not discolored.

IV. Physiology.

Aerobic

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	25°- 30°C.
Maximum temperature for growth	37°C.
Minimum temperature for growth	8°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., about pH 7.8 - 8.5.

Limits of pH for growth; from 5.0 to 11.0

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

17. Mycobacterium agreste.

I. Morphology. (Studied from B.P. Agar cultures at 37°C.).

Rods, 2 to 10 μ long by 0.5 to 1.0 μ broad, curved and occasionally branched. Longer forms, thread-like, up to 20 μ, which are more difficult to stain, are also found. There are also short "coccoid" bacilli, almost rectangular in shape, about 1.0 μ long by 0.5 to 1.0 μ broad. These coccoid bacilli occur lying in chains like chains of streptococci or strepto-bacilli, and seem to be a fragmentation of the longer rods. With some staining procedures, a very faintly staining sheath seems to hold them together. In older cultures, 2 weeks and older, although the occasional rod form is seen, the majority of bacilli appear in the form of these short coccoid cells. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). The longer rods, and "coccoid" forms stain intensely blue, distinctly Gram-positive. The very long thread-like rods stain very faintly, sometimes a distinct pink. (Pl. 15, fig. 1).

(Hucker). Gram-positive, as by the Kopeloff and Beerman technique. The cells, however, do not stain quite such an intense blue. (Pl. 15, fig. 2).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol-fast. The cells all stain blue. It is in this procedure that sometimes the coccus forms appear to be held together in a very indistinctly staining larger cell. (Pl. 15, fig. 3).

(Gabbet). In cultures, 24 and 48 hours old, the organisms are non-acid-fast. After this, acid-fast short rods appear among the non-acid-fast bacilli. The number of these acid-fast cells increases with the age of the culture. (Pl. 15, fig. 4 and 5).

Muir's Capsule Stain. (3 months on Glycerin Agar). The cells stain unevenly, appearing beaded. No definite capsules could be distinguished.

II. Cultural Characters. (at 37°C.).

Gelatin colonies. (10 days).

Surface colonies. About 1.2 m.m. in diameter; round; capitate; matte; edge finely erose; pink; internal structure, floccose with a few arborescent projections from the edge. No liquefaction.

Deep colonies. Much smaller, about 0.3 m.m. in diameter, round, pink.

Dextrose Agar colonies. (7 days).

Surface colonies. About 1.5 to 2.0 m.m. in diameter; round; raised with central knob and

radiate ridges; moist and shining; smooth terraced border; pale pink; on magnification, the edge appears undulate and very arborescent; internal structure, filamentous.

Deep colonies. "Burrs", with very arborescent edges.

Glycerin Agar Stroke. (48 hours). Good growth but not abundant; filiform; thin; flat; membranous-like; slightly moist; wrinkled; edge auriculate; light coral red (R). (10 days) Becoming raised; edge terraced and auriculate; closely wrinkled but growth not heaped up; coral red (R).

Dextrose Agar Stroke. (48 hours). Good growth, more abundant than on Glycerin Agar; slightly spreading; raised; moist; smooth; edge lobate; grenadine pink (R). (7 days) Becoming wrinkled, edge terraced with a distinct, pale arborescent fringe; pigment becomes deeper.(Pl. 30, fig.. 1. C).

Nutrient Agar Stroke. (48 hours). Good growth; filiform; raised; moist; smooth; lobate edge; flesh pink. (7 days) Becoming rough, with a paler almost transparent arborescent fringe.

Dorset's Egg Medium. (2 weeks). Good growth, very similar to the growth of Mycobacterium rubrum on this medium. Filiform; raised; dry; surface slightly wrinkled; apricot orange (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). A wrinkled, rather fragile, pink pellicle which is easily broken into large flakes, that settle to the bottom of the flask. Liquid clear.(Pl.33,fig. 2.B).

Loeffler's Blood Serum. (48 hours). Good growth; filiform to spreading; raised; moist; smooth; pink. (7 days) Becoming carrot red (R) and granular. No digestion or liquefaction of the medium.

Glycerin Potato. (48 hours). Good growth; filiform; raised; thick; dull; dry; wrinkled; yellowish brown turning coral red (R).

Gelatin Stab. Pink, raised, surface growth. Beaded growth in stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Reddish pellicle growth. Alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose . Acid formed in 1% Glycerin after 14 days.

Final Reaction in 5% Glycerin Broth.(4 weeks). pH 5.4.

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brownish surface growth. No growth in butt. No discoloration of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	37°C.
Maximum temperature for growth	48°C.
Minimum temperature for growth	8°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc., for growth; about pH 6.8 to 8.0

Limits of pH for growth; from 5.0 to 11.0

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

18. Mycobacterium album.

I. Morphology. (Studied from B.P.Agar cultures at 25°C.).

Rods, varying in size from 1.0 to 5.0 μ in length by 0.3 to 0.6 μ wide; majority about 2.0 μ long. Straight or slightly curved. The rods are about 3 to 4.0 μ long in young cultures, 24 hours old, but in cultures, 48 hours old and older, the rods are rarely longer than 2.0 μ. No very long or branched forms were observed. The cells lie singly but more commonly in parallel arrangements. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Distinctly Gram-positive, the cells staining a bright blue. (Pl. 16, figs. 1 and 2).

(Hucker). As with the Kopeloff and Beerman technique. Gram-positive. (Pl.16, figs. 3 & 4).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol-fast. The organisms stain a very pale blue. (Pl. 16, figs. 5 and 6).

(Gabbet). In young cultures, 24 and 48 hours old, not acid-fast. In 7 day old cultures and cultures older than this a few cells stain acid-fast. (Pl. 16, figs. 7 and 8).

Muir's Capsule Stain. (1 month on Glycerin Agar). No distinct capsules could be distinguished.

II. Cultural Characters. (at 25°C.).

Gelatin colonies. (10 days).

Surface colonies. About 1.0 m.m. in diameter; round; raised; dry; squamose; erose; grayish; internal structure "curled". Not liquified.

Deep colonies. Very small; round; white; about 0.3 m.m. in diameter.

Dextrose Agar. (7 days).

Surface colonies. Very small; about 1.0 m.m. in diameter; round; slightly raised; grayish-white; on magnification the edge is a mass of long arborescent projections; internal structure, filamentous.

Deep colonies. Very minute.

Glycerin Agar Stroke. (48 hours). Good growth; slightly spreading; thin; very slightly moist; finely granular (not smooth); edge somewhat like that of B. anthracis, irregular, erose and "fuzzy"; grayish-white.

Dextrose Agar Stroke. Growth similar and as abundant as on Glycerin Agar. (Pl. 30, fig. 2. A).

Nutrient Agar Stroke. (48 hours). Growth not as good as on Glycerin or Dextrose Agar; filiform; flat; thin; very slightly moist; dull; granular; edge erose; grayish-white.

Dorset's Egg Medium. (2 weeks). Scanty growth; spreading; thin; dry; sea-shell pink (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks) Thin, fragile, grayish-white, granular pellicle which climbs up the sides of the flask. Slightly turbid with small grayish sediment.

Loeffler's Blood Serum. (1 week) Good growth; filiform; raised; slightly moist; granularly rough; cream (R). No liquefaction or digestion of the medium.

Glycerin Potato. (24 hours) Grayish-white, raised, dry, granular growth, becoming (in 7 days) pale buff.

Gelatin Stab. Grayish, raised, surface growth. Filiform growth in stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Grayish pellicle. Developing a slight alkalinity in 9 days. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 5.4

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not Hydrolyzed.

Lead Acetate Agar. Brown surface growth. No growth in butt. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	25°- 30°C.
Maximum temperature for growth	35°C.
Minimum temperature for growth	10°C.

Relation to Reaction of the Medium (5% Glycerin Broth).

Optimum H-ion conc., for growth; about pH 7.8 - 8.5

Limits of pH for growth; from 4.4 to 11.2.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

19. Mycobacterium coeliacum.

I. Morphology. (Studied from B.P. Agar at 25°C.).

Rods, 2 to 12 μ long by 0.5 to 1.0 μ wide; majority 4 to 7 μ long by about 0.7 μ wide. Curved and branching. Often unevenly swollen, club-shaped. Coccoid forms also present, about 0.7 μ in diameter, occurring in chains like "streptococci". The long rods, very numerous in young cultures, decrease in number with age, while the "coccoid" forms increase. The staining characteristics are not in any way altered with the period of incubation. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Rods and cocci stain intensely blue. Gram-positive. (Pl. 17, figs. 1 and 2).

(Hucker). As with the above technique, the cells are Gram-positive. The bacilli, however, are not as deeply stained or as thick as in the preparations stained by the Kopeloff and Beerman technique. (Pl. 17, figs. 3 & 4).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol-fast. Rods and cocci stain a pale blue. (Pl. 17, fig. 5).

(Gabbet). Not acid-fast.

(Pl. 17, fig. 6).

Muir's Capsule Stain. (3 months on Glycerin Agar). No individual capsules could be demonstrated, but numerous bacilli were held together in a mass of gummy-like material.

II. Cultural Characters. (at 25°C.).

Gelatin colonies. (10 days).

Surface colonies. Small; about 1.5 m.m. in diameter; irregularly round, sometimes conglomerate; capitate, some colonies almost pointed; surface segmented; lobulate; very pale buff; internal structure, floccose. No liquefaction.

Deep colonies. Smaller; 0.5 m.m. or less in diameter; irregular, often appearing "star-shaped".

Dextrose Agar colonies (7 days).

Surface colonies. 1.0 to 3.0 m.m. in diameter; irregularly round; raised; moist; smooth; shining; surface concentrically ringed and umbonate; grayish-white. (13 days) Colonies larger 4 - 5 m.m. in diameter; surface raised and concentrically ringed; moist; shining; border radiately segmented; edge lobate and burred; cartridge buff (R); internal structure, filamentous.

Deep colonies. Very irregular, edge sometimes deeply lobate; smaller than surface colonies; pale pink becoming cartridge buff (R).

Glycerin Agar Stroke. (48 hours). Good growth; spreading; raised; slightly moist and wax-like; wrinkled; smooth; concentrically ringed; edge lobate and tufted; flesh colored becoming cartridge buff (R).

Dextrose Agar Stroke. Growth similar and as abundant as on Glycerin Agar. **(Pl. 28, fig. 1. C).**

Nutrient Agar Stroke. Growth not as abundant or as rapid as on Glycerin or on Dextrose Agar; filiform; smooth; moist; finely granular; edge undulate; grayish.

Dorset's Egg Medium. (2 weeks) Good growth. Isolated colonies, round, convex, smooth, moist, becoming in the drier part of the slant, slightly dry and shining with a depression in the centre. When more numerous they coalesce to form a thick, smooth, verrucose, pale ochraceous buff (R) streak. Medium not darkened or digested.

Glycerin Broth. (3 days in flasks) Turbid with pale pink, fragile, dry, wrinkled pellicle. Sour odor.

Loeffler's Blood Serum. (7 days). Moderate growth; filiform; slightly raised; dry; granular; pale ochraceous buff (R). No digestion or liquefaction of the medium.

Glycerin Potato. (48 hours). Rapid, good growth; thick; raised; dry; dull; granular; flesh colored.

Gelatin Stab. Flesh colored, raised, surface growth. Scanty, filmy growth in stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Grayish pellicle. After 10 days becoming slightly alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth.(4 weeks). pH 5.2.

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brown surface growth. No growth in the butt. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	25°C.
Maximum temperature for growth.	35°C.
Minimum temperature for growth	8°C.

Relation to Reaction of the Medium (5% Glycerin Broth).

Optinum H-ion conc. for growth; about pH 7.1 - 7.3.

Limits of pH for growth; from 5.0 to 11.2.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

20. Mycobacterium convolutum.

I. Morphology. (Studied from B.P. Agar cultures at 25°C.).

Rods, long, curved and filamentous. In young cultures, 1 and 2 days old, besides the shorter bacilli, 4.0 to 10.0 μ long, there are many very long, thick, curved and branching rods, 25 to 40.0 μ long by 0.5 to 0.7 μ wide. Short, thicker rods, 3.0 to 4.0 μ long by 0.7 to 1.0 μ wide are also present. As the culture ages, the short coccoid bacilli become more numerous. After 2 weeks growth, the cultures are composed almost entirely of these short thick rods and cocci. The long rods lie singly, but more often in tangled masses, the coccoid forms are arranged in chains of varying lengths. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain.(Kopeloff and Beerman). The cells stain distinctly Gram-positive by this procedure, in young and old cultures. (Pl. 18, figs. 1 and 2).

(Hucker). Weakly Gram-positive. After 2 weeks, the greater number of the short rods and cocci do not stain blue by this procedure. (Pl. 18, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol-fast. The bacilli stain a faint blue. (Pl. 18, figs. 5 & 6).

(Gabbet). Not acid-fast in young

cultures. In old cultures (2 weeks and older), there appear some distinctly acid-fast cocci or granules. (Pl. 18, figs. 7 and 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). A very faintly staining delicate capsule surrounds the individual cells. Clumps of bacilli lie grouped together in a mass of gummy-like material.

II. Cultural Characters. (at 25°C.).

Gelatin colonies. (10 days).

Surface colonies. 1.5 - 2 m.m. in diameter; irregularly round; raised; matte; slightly ridged; edge filamentous; pale yellow; internal structure, floccose with arborescent projections from edge. No liquefaction.

Deep colonies. Much smaller, 0.5 m.m. in diameter, round.

Dextrose Agar colonies. (7 days).

Surface colonies. 3.0 to 5.0 m.m. in diameter; irregularly round; raised; dry; surface segmented in rosette formation; edge lobate and filamentous; grayish-white turning pale yellowish; internal structure, filamentous.

Deep colonies. Smaller, circular, pale yellow. burrs.

Glycerin Agar Stroke. (48 hours). Abundant growth; spreading; raised; thick; dry; rough, broken into a number of segments; edge lobate; flesh-colored. (Pl. 29, fig. 2. B).

Dextrose Agar Stroke. More abundant than on Glycerin Agar. (Pl. 29, fig. 1. B).

Nutrient Agar Stroke. (7 days). Moderate growth; filiform; raised; slightly moist; uneven edge; pale pink.

Dorset's Egg Medium. (2 weeks). Good growth. Isolated colonies round, smooth, moist, coalescing to form a thich, raised, verrucose, buff-colored streak. Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Thick, opaque, grayish, rough, adherent pellicle, which climbs part way up the sides of the flask. Medium clear with flaky sediment. Sour odor. (Pl. 33, fig. 1. A).

Loeffler's Blood Serum. (7 days). No growth. (10 days). Growth as discreet isolated colonies, raised, almost umbonate, dry, granular, pale ochraceous buff (R). No digestion or liquefaction of the medium.

Glycerin Potato. (2 days). Dry, crumpled, orange, becoming in 10 days pecan brown (R).

Gelatin Stab. (7 days). Whitish surface growth. Scanty growth along stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Becoming in 7 days slightly alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 5.2.

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brownish surface growth. No growth in the butt. Medium not blackened.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth 25°- 30°C.

Maximum temperature for growth 37°C.

Minimum temperature for growth 8°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc.for growth; about pH 7.6 - 8.0

Limits of pH for growth; from 5.0 to 10.8.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

21. Mycobacterium crystallophagum.

I. Morphology. (Studied from B.P.Agar cultures at 30°C.).

Rods, varying in size from 3.0 to 30.0 μ long by 0.4 to 0.8 μ wide; majority 3.0 to 8.0 μ long by about 0.5 μ wide; curved and branching; sometimes unevenly swollen but generally of an even thickness. Coccoid forms about 1.0 u long present in young and older cultures; these coccoid rods are generally almost rectangular in shape and occur lying in short chains; they are far more numerous in old than in young cultures, while the long rods decrease in number with age. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Most of the rods and cocci stain an intense blue; distinctly Gram-positive. Some of the longer filamentous forms do not stain by Gram; they take a pink coloration. (Pl. 19, figs. 1 and 2).

(Hucker). As with the above technique, the rods and cocci stain blue, with a few cells staining very weakly. The organisms appear more slender by this staining procedure than by the Kopeloff and Beerman stain. (Pl. 19, figs. 3 & 4).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol-fast. Rods and cocci stain a pale blue. (Pl. 19, figs. 5 and 6).

(Gabbet). The rod forms and most of the cocci stain blue but there are a few coccoid cells which are distinctly acid-fast, staining red. These acid-fast cells are present in cultures, 24 hours and 1 month old, but are more numerous in the old cultures than in the young. (Pl.19, figs. 7 and 8).

Muir's Capsule Stain.(3 months on Glycerin Agar). The cells stain unevenly, appearing beaded, with deeply staining polar bodies. No definite capsular material could be distinguished

II. Cultural Characters. (at 30°C.).

Gelatin colonies. (10 days).

Surface colonies. Small; 1.0 to 1.5 m.m. in diameter; round; capitate; matte; edge entire in the smaller colonies, but finely erose in the larger colonies; internal structure, filamentous, the edges appearing "burred". No liquefaction.

Deep colonies. Very small, about 0.3 m.m. in diameter, round, entire.

Dextrose Agar colonies. (7 days).

Surface colonies. 1.0 to 5.0 m.m. in diameter; round; moist and glistening, tending to become dry; sea-shell pink. Small colonies convex and smooth. Larger colonies raised in the centre but flattening out towards the edge, surface

marked by concentric and radiate lines. On magnification, a paler fringe undulate and filamentous, is seen. Internal structure, filamentous.

Deep colonies. Irregularly round, edge filamentous and having numerous arborescent projections.

Glycerin Agar Stroke. (48 hours). Good growth; filiform to spreading; slightly moist; smooth; edge undulate; grayish-white. (2 weeks) Becoming spreading; dry and powdery; surface wrinkled and slightly heaped up at the bottom of the slant; edge more definitely lobate; light buff (R).

Dextrose Agar Stroke. Growth similar and as abundant as on Glycerin. **(Pl. 28, fig. 1. A).**

Nutrient Agar Stroke. Growth not as abundant or as rapid as on Glycerin or Dextrose agar; filiform; slightly moist; surface at first smooth becoming granular; edge undulate becoming tufted; grayish-white.

Dorset's Egg Medium. (2 weeks). Good growth; spreading; moist; surface smooth but verrucose; pale ochraceous salmon (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Pink, dry, fragile pellicle which becomes thicker and

slightly wrinkled. It is, however, easily broken. Liquid clear, but soon made turbid by the settling of the flakes from the broken pellicle.(Pl. 33, fig. 2. D).

Loeffler's Blood Serum. (7 days). Poor growth. Isolated colonies round; raised; umbilicate; slightly moist; light buff (R). No liquefaction or digestion of the medium.

Glycerin Potato. (24 hours). Dry; rough; crumpled; sea-shell pink. (5 days) Becoming cinnamon buff (R).

Gelatin Stab. Grayish, convex, smooth surface growth. Filiform growth in stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Grayish pellicle. Slight alkalinity developed in 4 days. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth.(4 weeks) pH 6.0.

Nitrate Broth and Agar. Nitrates reduced to nitrites after 4 days growth on Nitrate Agar. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brownish surface growth. No growth in the butt. Medium not blackened.

IV. Physiology.

Aerobic

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	30°C.
Maximum temperature for growth	37°C.
Minimum temperature for growth	8°C.

Relation to Reaction of the Medium.(5% Glycerin Broth).

Optimum H-ion conc. for growth; about pH 6.8 - 7.3.

Limits fo pH for growth; from 4.4 to 11.0.

Indol Agar. Indigotin not formed.

Blood Agar Plate. Blood haemolyzed. A distinct clear zone with slight greenish coloration is formed around the area of growth.

22. Mycobacterium erythropolis.

I. Morphology. (Studied from B.P. Agar cultures at 25°C.).

Rods, varying in size from 2.5 to 18.0 μ long by 0.4 to 0.75 μ wide; majority 4.0 to 8.0 μ long by about 0.5 μ wide. Curved and branching forms present in young culture (24 hours); there are also numerous coccoid forms 0.5 to 1.0 μ long and some very minute "dots". The large rods, though often of even thickness, are more often swollen unevenly at the end or ends, and sometimes in the middle. The ends are rounded. The "dots" and "coccoid" forms are generally arranged in pairs like "diplococci" and in very short chains. The longer forms are more numerous in young culture and decrease with age; the "coccoid" form increases considerably in number with the age of the culture. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Majority of rods stain deeply Gram-positive, but a number also stain pink or mauve. The coccoid forms stain more deeply. (Pl. 20, figs. 1 and 2).

(Hucker). The cells do not stain such an intense blue as by the Kopeloff and Beerman technique, and do not appear quite as thick. There are also present many weakly staining cells, and cells which stain a faint pink. (Pl.20, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). Distinctly non-acid-alcohol-fast. (Pl. 20, figs. 5 and 6).

(Gabbet). Non-acid-fast. (Pl. 20, figs. 7 and 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). The cells stain very unevenly; distinctly bipolarly. Cells when in clumps seem to be held together by faintly staining gummy-like material.

II. Cultural Characters. (at 25 C.).

Gelatin colonies. (10 days).

Surface colonies. About 2.0 m.m. in diameter; round; pulvinate; matte; edge entire, on magnification, undulate;very pale buff; internal structure, floccose, the edge fringed with a mass of finely woven threads and arborescent projections; 0.3 m.m. in diameter.

Deep colonies. Similar but much smaller; 0.3 m.m. in diameter, round.

Dextrose Agar colonies. (7 days).

Surface colonies. 2.0 to 3.0 m.m.in diameter; round; convex and sometimes umbonate showing a few concentric markings; moist; shining; edge unevenly undulate (when magnified); pale flesh colored; internal structure granular.

Deep colonies. Smaller, pale pink, very irregular.

Glycerin Agar Stroke. (2 days). Good growth; spreading; flat; dry; surface coarsely granular; edge undulate, becoming lobate and fringed with a pale transparent almost arborescent border. bittersweet pink (R). In some cultures the growth is thicker, raised, and segmented, almost alveolate; easily emulsified.

Dextrose Agar Stroke. Similar to Glycerin Agar stroke culture. Growth somewhat more rapid and abundant, with a little deeper pigmentation. (Pl. 29, fig. 1. D).

Nutrient Agar Stroke. (3 days). Slower growth and not as abundant as on Glycerin and Dextrose Agar. Isolated colonies, very small, slightly raised, round; grayish, coalescing to form a granular, very pale pink streak.

Dorset's Egg Medium. (2 weeks). Good growth; spreading; slightly raised; moist; finely granular; pale wavy fringe; flesh colored (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Pale pink, wrinkled, flaky pellicle which is not very adherent but is easily broken. Slight turbidity.

Loeffler's Blood Serum. (2 days). Very scanty, hardly visible growth. (7 days) Good growth; slightly spreading; raised; slightly moist; granular; sea-shell pink (R). Isolated colonies, very small, round, smooth, moist, pale pink. No digestion or liquefaction of the medium.

Glycerin Potato. (2 days). Poor growth.

(7 days) Slightly spreading; dry; rough and granular; almost flat; orange rufous (R).

Gelatin Stab. Pinkish, raised, convex surface growth. Whitish filiform growth. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Pale pink pellicle. Alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 5.2.

Nitrate Broth and Agar. Nitrates reduced to nitrites after 7 days. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brownish surface growth. No growth in the butt. Medium not blackened.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	25°C.
Maximum temperature for growth	35°C.
Minimum temperature for growth	8°C.

Relation to Reaction of the Medium.(5% Glycerin Broth).

Optimum H-ion conc. for growth; about pH 6.8 - 8.0

Limits of pH for growth; from 5.0 to 11.2.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

23. Mycobacterium globerulum.

I. Morphology. (Studied from B.P. Agar cultures at 28°C.).

Rods, varying in size from 1.0 to 15.0 μ long by 0.2 to 1.0 μ wide. This micro-organism is very pleomorphic: there are short slender rods 3.0 to 6.0 μ long by about 0.3 μ wide, thicker bacilli 3.0 to 6.0 μ long by about 1.0 μ wide, cocci about 1.0 μ wide and longer thread-like forms up to 15 μ long. The bacilli and long thread-like forms are more numerous in young cultures, 24 hours old, than older cultures: the coccoid forms, which are not very numerous in young cultures, increase greatly in numbers with the age of the culture. The rod forms lie singly and in masses; the cocci are arranged in pairs, in short chains and in irregular clusters. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Distinctly Gram-positive, in young and old cultures, all the forms staining intensely. (Pl. 21, figs. 1 and 2).

(Hucker). As with the Kopeloff and Beerman technique, all the forms stain distinctly Gram-positive. (Pl. 21. figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). The organisms stain very weakly. None of the forms are acid-alcohol-fast. (Pl. 21, figs. 5 and 6).

(Gabbet). Not acid-fast in young or older cultures. (Pl. 21, figs. 7 and 8).

Muir's Capsule Stain. (24 hours on Dextrose Agar). The organism forms a wide capsule on this medium. These capsules surround not only the individual cell but often a group of cells.

These capsules also formed in older cultures on B.P. Agar are easily demonstrated by the capsule stains of Muir and of Hiss.

II. Cultural Characters. (at 28°C.).

Gelatin colonies.(10 days).

Surface colonies. 1 - 2 m.m. in diameter; round; convex; smooth and shining; moist; edge entire; buff colored; internal structure, coarsely granular. No liquefaction.

Deep colonies. Much smaller, round, entire.

Dextrose Agar colonies. (7 days).

Surface colonies. 2.0 to 5.0 m.m. in diameter; round; convex; moist; shining; smooth but showing a slight marking of concentric rings; pale pink; internal structure, filamentous.

Deep colonies. Smaller, very irregularly shaped.

Glycerin Agar Stroke. (24 hours). Good growth; filiform; raised; moist and glistening; smooth; grayish-white. (7 days) Becoming spreading; thicker; very moist; sea-shell pink (R). Viscous.

Dextrose Agar Stroke. More abundant, thicker, more moist and deeper pink than on Glycerin Agar. (Pl. 30, fig. 2 B).

Nutrient Agar Stroke. (24 hours). Filiform, grayish-white becoming pink similar to Glycerin Agar slant only more limited growth

Dorset's Egg Medium. (2 weeks). Good growth; spreading; raised; very moist and smooth; Capucine Orange (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). No pellicle, slightly cloudy with a few masses of thicker suspended floccules. After 5 days, a very slight filmy pellicle forms and the medium becomes very turbid. (Pl. 32, fig. 2. B).

Loeffler's Blood Serum. (48 hours). Smooth, moist, glistening, raised, round, shell pink (R) colonies. Becoming, in 7 days, a thick, confluent, moist, smooth, salmon colored (R) streak. No liquefaction or digestion of the medium.

Glycerin Potato. (24 hours). Filiform; moist; smooth; pale pink. (9days) Abundant; raised; apricot buff (R).

Gelatin Stab. (9 days). Thick, raised, smooth, moist, pale pink surface growth. Filiform growth in medium. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Alkaline. Very slightly peptonized in 2 weeks. No coagulation.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose or Sucrose.

Acid formed, in 2 days, in 1% Glycerin Broth.

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 5.0.

Nitrate Broth and Agar. Nitrates reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Grayish surface growth. No growth in the butt. No blackening or discoloration of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	28°C.
Maximum temperature for growth	37°C.
Minimum temperature for growth	10°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc. for growth; about pH 6.8 - 7.6.

Limits of pH for growth; from 5.0 to 11.0

Indol Agar. Colonies (3 days) Round and blue. On

magnification, small bluish crystals are seen projecting from the colony into the medium.

Stroke. (3 days) Filiform, smooth, moist growth, with thicker, bluish portions due to the production of Indigotin.

Blood Agar Plate. No haemolysis.

24. Mycobacterium hyalinum.

I. Morphology. (Studied from B.P. Agar cultures at 20°C.).

Rods, very minute, varying in size from 0.3 μ to 2.0 μ long by 0.25 μ and less in width, some of the organisms appearing no larger than dots. The rods are straight or slightly curved. No longer thread-like, or branched forms were observed. The rods lie singly, sometimes in parallel formation. The morphological appearance is the same in cultures, 24 hours old and 30 days old. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Weakly Gram-positive in 24 hour old cultures, but in 48 hour old cultures and cultures older, the majority of cells stain distinctly Gram-positive, with only a few cells not staining, or staining pink. (Pl. 22, fig. 1.).

(Hucker). Weakly Gram-positive. (Pl. 22, fig. 2.).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol-fast in young or old cultures. (Pl. 22, fig. 3.).

(Gabbet). Non-acid-fast. The bacilli stain a pale blue. (Pl. 22, fig. 4.).

Muir's Capsule Stain. (3 months on Glycerin Agar). No distinct capsules could be distinguished, but a faintly staining gummy material seemed to hold together masses of the cells.

II. Cultural Characters

Gelatin colonies.(10 days).

Surface colonies. Very small; about 0.5 m.m. in diameter; round; slightly convex; moist; smooth; entire edge; pale glass green; internal structure, amorphous.

Deep colonies. "Pin-points."

Dextrose Agar colonies (7 days).

Surface colonies. From 0.5 to 1.0 m.m. in diameter; round; slightly convex; moist; smooth; edge entire; white turning pale greenish yellow; internal structure, clear, amorphous.

Deep colonies. Small; lens-shaped; edge entire.

Glycerin Agar Stroke. (48 hours). Good growth; spreading; slightly raised almost flat; moist; glistening; smooth; entire edge; grayish-white; transparent at first, becoming opaque. Very viscous in old culture.

Dextrose Agar Stroke, (24 hours). Filiform; thin; moist; glistening; smooth; grayish. (5 days) Becoming spreading; entire edge; light green-yellow.

Nutrient Agar Stroke. Growth as good as on Dextrose or Glycerin Agar.

Dorset's Egg Medium. (2 weeks). Good growth; filiform; moist; glistening; smooth; massicot yellow (R). Medium not darkened or digested.

Glycerin Broth.(3 days in flasks). No pellicle formation. Turbid with pale greenish sediment. (Pl. 32, fig. 1. C).

Loeffler's Blood Serum. (48 hours). No growth. (7 days) Good growth; spreading; moist; smooth; pale green; liquefying the medium.

Glycerin Potato. (48 hours). Hardly visible growth. Very small, moist, glistening, pale yellow, isolated colonies. (2 weeks) Filiform; flat; moist; smooth; edge entire; chamois (R).

Gelatin Stab. Greenish yellow surface growth. Filiform growth along line of inoculation. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. No change.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose or Sucrose.

Acid formed, in 7 days, in 1% Glycerin Broth.

Final Reaction in 5% Glycerin Broth.(4 weeks). pH 5.2.

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. No growth in the butt. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	20°C.
Maximum temperature for growth	35°C.
Minimum temperature for growth	8°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc. for growth; about pH 7.1 - 8.5.

Limits of pH for growth; from 5.2 - 11.2.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

25. A. Mycobacterium lacticola.

I. Morphology. (Studied from B.P. Agar cultures at 25°C.).

Rods, varying in size from 1.0 μ to 15.0 μ long by 0.35 to 0.8 μ wide. Majority of cells in cultures, 24 hours old, are 3.0 μ to 6.0 μ long by 0.5 μ wide, with the occasional long thread-like form. Rods are straight or slightly curved, sometimes swollen at the ends giving a club-shaped appearance to the cell, and sometimes though less frequently, swollen in the middle. Branching very frequently occurs. Besides the rod shaped cells, there is also a short, thick coccoid-like rod, occurring in young cultures though more frequently in older cultures. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). All the rods and coccoid bacilli stain very intensely in young and old cultures. The bacilli appear much thicker by this stain than by the Hucker modification.

(Pl. 23, figs. 1 and 2).

(Hucker). The longer rods and most of the shorter ones stain weakly by Gram, both in young cultures and cultures a month old. The shorter coccoid-like bacilli and certain granule-like portions of the cell, however, stain deeply, giving the appearance of a beaded rod.

(Pl. 23, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). Non-acid alcohol-fast. In very young cultures, 24 hours old and younger, a few cells stain pink but in cultures, 48 hours old, and cultures older than this, the bacilli are distinctly non-acid-alcohol-fast. This staining technique, however, shows up quite distinctly the granular nature of the bacilli, the granules staining a bright blue in a faintly stained cell. (Pl. 23, figs. 5 and 6).

(Gabbet). As with the Ziehl-Neelsen technique, in very young culture a few of the bacilli stain pink, appearing acid-fast. This property is very soon lost, however, and the organisms stain an even very pale blue. (Pl. 23, figs. 7 and 8).

Muir's Capsule Stain. (1 month on Glycerin Agar). Distinct, sometimes wide capsules surround the individual cells and coccoid forms. Clumps of bacteria lie together in a mass of capsular material.

II. Cultural Characters (at 25°C.).

Gelatin colonies. (10 days).

Surface colonies. 2.0 to 3.0 m.m. in diameter; round; capitate; some colonies, smooth and moist; others dry and matte; lobulate; pale buff;

internal structure, floccose, with a fringe of arborescent projections. No liquefaction.

Deep colonies. Similar to surface colonies, but much smaller; about 0.5 m.m. in diameter; round; entire.

Dextrose Agar colonies. (7 days).

Surface colonies. About 1 m.m. in diameter; round; convex; slightly moist and glistening; smooth; pale ochraceous-salmon (R). Under low magnification, the edge appears "burr-like".

Deep colonies. Small; irregular; cream-colored. The arborescent and filamentous nature of the edge is more pronounced.

Glycerin Agar Stroke (48 hours). Abundant growth; spreading; thick; very moist; slightly roughened, becoming smooth and slimy; sea-shell pink (R).

Dextrose Agar Stroke. (48 hours). Very abundant growth; thick and moist like Glycerin Agar Stroke. (Pl. 30, fig. 2. D).

Nutrient Agar Stroke. (48 hours). Good growth, but not as good as on Dextrose or Glycerin Agar; slightly spreading; very slightly raised; almost flat; moist; sea-shell pink.

Dorset's Egg Medium. (2 weeks). Good growth; spreading; raised, very moist and smooth; capucine buff (R). Medium not blackened nor digested.

Glycerin Broth. (3 days in flasks). Thin, pinkish, opaque, flaky pellicle, not very adherent. With age, the pellicle gets somewhat thicker, but large flakes break away and settle very slowly, causing a rather thick flaky turbidity in the medium.

Loeffler's Blood Serum. No visible growth at the end of three days. In seven days, the culture had grown well. Spreading; moist; smooth; verrucose; pale salmon color (R). No digestion or liquefaction of the medium.

Glycerin Potato. (48 hours). Little growth, and slightly spreading, dry. (10 days) Thick, rough. Light-salmon orange (R).

Gelatin Stab. Whitish growth at surface; very scanty along line of inoculation. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Pinkish pellicle. Becoming slightly alkaline in 10 days. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose ro Glycerin (1%).

Final Reaction in 5% Glycerin Broth(4 weeks). pH 5.2.

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Flesh colored surface growth.

No growth in the butt. No blackening of the medium.

IV. Physiology.

Aerobic

Relation to Temperature. (on Dextrose Agar).

Optimum temperature for growth	25°C.
Maximum temperature for growth	37°C.
Minimum temperature for growth	8°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc. for growth; about pH 6.8 - 7.8

Limits of pH for growth; from 5.0 to 11.2.

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

25. B. Mycobacterium lacticola.

This organism was studied with the hope of its being Mycobacterium friburgensis, but it was non-acid-fast and identical in its morphology and all its cultural characteristics with Mycobacterium lacticola No. 25 A. previously described.

26. Mycobacterium luteum.

I. Morphology. (Studied from B.P. Agar cultures at 25°C.).

Rods, very short and slender, 0.9 to 3.0 μ long by 0.2 to 0.5 μ wide; majority of cells about 1.5 to 2.0 μ long by 0.3 μ wide. The bacilli are straight or slightly curved and generally of an even thickness. No longer thread-like or branched forms were observed in any of the cultures. The parallel arrangement of the organisms is very marked; the appearance and arrangement of the organisms is very similar to that of Mycobacterium marinum.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). The organisms stain an intense blue being distinctly Gram-positive in cultures, 24 hours old. In cultures, a week and 2 weeks old, the Gram-positivity is markedly weaker, a large number of the cells staining but weakly and some cells staining unevenly, giving a beaded appearance. (Pl. 24, fig. 1.).

(Hucker). As with Kopeloff and Beerman's stain, the organisms are Gram-positive in young culture, but gradually become weaker in this characteristic, a number of the cells in 2 week old cultures staining a very faint pink.(Pl. 24, fig. 2.).

Acid-Fast Stain.(Ziehl-Neelsen). Not acid-alcohol-

fast. The cells stain a very pale blue in cultures, 24 hours and 2 weeks old. (Pl.24, fig. 3.).

(Gabbet). Not acid-fast. The cells stain similarly by this and the Ziehl-Neelsen technique. (Pl. 24, fig. 4.).

Muir's Capsule Stain. (2 months on Glycerin Agar). The cells stain very unevenly. No distinct capsules surrounding the cells could be distinguished.

II. Cultural Characters. (at 25°C.).

Gelatin colonies (10 days).

Surface colonies. 1.5 to 2.0 m.m. in diameter; round; capitate; smooth; entire; yellow; internal structure, floccose. No liquefaction.

Deep colonies. Much smaller; round; about 0.5 m.m. in diameter.

Dextrose Agar colonies. (7 days).

Surface colonies. Very small; 0.5 to 1.0 m.m. in diameter; round; moist; glistening; smooth; edge entire; orange; internal structure, coarsely granular.

Deep colonies. Irregularly round; lens-shaped; orange.

Glycerin Agar Stroke. (2 days). Very poor growth. (7 days) Good growth; raised; thick; slightly moist and glistening; smooth becoming coarsely granular and then finely wrinkled; edge undulate; orange

buff (R). Very viscous in old culture.

Dextrose Agar Stroke. (7 days) Good growth; spreading; moist; at first smooth becoming finely granular; edge undulate; orange. Deeper pigmentation than on Glycerin Agar at first, but with age the deep pigment also develops on the Glycerin Agar. (Pl. 29, fig. 1. C).

Nutrient Agar Stroke. Similar to growth on Glycerin or Dextrose Agar but no as abundant. Lighter pigmentation.

Dorset's Egg Medium. (2 weeks) Good growth, filiform; raised; smooth; orange (R). Medium not darkened or digested.

Glycerin Broth. (7 days in flasks). Very thin, fragile, flaky yellowish pellicle. Turbid with thick, viscous, orange sediment.(Pl. 33, fig. 1. C).

Loeffler's Blood Serum. (7 days) Good growth. Isolated colonies, small, raised, convex, smooth. When more numerous, forming a moist, granular streak; Orange (R). No digestion or liquefaction of the medium.

Glycerin Potato. (2 days). Poor growth; moist; slightly raised; orange (R); becoming thicker and slightly roughened.

Gelatin Stab. Moist, orange surface growth. Filiform orange growth on stab. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose, Sucrose or Glycerin (1%).

Final Reaction in 5% Glycerin Broth. (4 weeks). pH 6.8.

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar Dark brown surface growth. No growth in the butt. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature .(on Dextrose Agar).

Optimum temperature for growth	25°C.
Maximum temperature for growth	37°C.
Minimum temperature for growth	8°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion conc. for growth; about pH 6.6.

Limits of pH for growth; from 5.0 to 11.0

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

27. Mycobacterium opacum.

I. Morphology. (Studied from B.P. Agar cultures at 30°C.).

Rods, ranging in size from 2.0 to 25.0 μ long by 0.5 to 1.0 μ wide. Majority 6.0 - 8.0 μ long. Very long rods curved and branching. There are also small coccoid cells about 0.5 to 1.0 μ in diamter, which are few in young cultures, 24 hours old. In older cultures, (7 days), the greater majority of the rods have broken into these small coccoid forms, which are arranged in short chains like streptococci. The bacilli are irregularly grouped together like a tangled mass of threads. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (Kopeloff and Beerman). Gram-positive. The bacilli and cocci stain intensely. (Pl.25, figs.1 & 2).

(Hucker). Gram-positive, not staining as deeply as by the Kopeloff and Beerman technique. (Pl. 25, figs. 3 and 4).

Acid-Fast-Stain. (Ziehl-Neelsen). Non-acid-alcohol-fast. (Pl. 25, figs. 5 and 6).

(Gabbet). Non-acid-fast. The occasional acid-fast rod or portion of a rod occurs lying in a mass of blue cells, in older cultures (2 weeks). (Pl.25, figs. 7 and 8).

Muir's Capsule Stain. (3 months on Glycerin Agar). The cells stain very unevenly, sometimes distinctly bipolarly. No individual distinct capsular material could be distinguished.

II. Cultural Characters. (at 30°C.).

Gelatin colonies. (10 days).

Surface colonies. Small; about 1.0 m.m. in diameter; round; capitate; matte; edge very finely lobulate; white; internal structure, floccose, the edge fringed with a thick mass of arborescent projections. No liquefaction.

Deep colonies. Much smaller; round; white; about 0.4 m.m. in diameter.

Dextrose Agar colonies. (7 days).

Surface colonies. Very small; less than 1.0 m.m. in diameter; round; convex; smooth; edge appears entire to the naked eye, but under magnification shows a mass of fine threads, "burr-like"; light buff. After 1 month, the edge is very arborescent, the arborescence being easily visible to the naked eye.

Deep colonies. Smaller; "burrs".

Glycerin Agar Stroke. (48 hours). Good growth; spreading; raised; moist; thick; slightly roughened surface; buff orange. (3 weeks). Slightly moist almost dry; surface verrucose with thick warty prominences; edge becoming tufted; orange.

Dextrose Agar Stroke. (48 hours). Abundant; spreading; raised; thick; moist growth; rough with definite markings; pale ochraceous buff (R).(Pl. 30, fig. 1. B).

Nutrient Agar Stroke. (48 hours). Scanty growth; spreading; moist; glistening. Growth not evenly smooth but consisting of a mass of dense, convex, smooth small colonies, at first white becoming pale ochraceous buff (R).

Dorset's Egg Medium. (2 weeks). Filiform to slightly spreading; raised; dry; surface roughened by small wrinkles. Isolated colonies, dry, raised, umbilicate, pale ochraceous buff (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Thin, grayish-white, opaque pellice, not wrinkled, easily breaking into flocculi which cause turbidity and a flocculent precipitate. (Pl. 32, fig. 1. A).

Loeffler's Blood Serum. (48 hours). Scanty, sea-shell pink growth. (7 days) Good growth; slightly spreading; dry; roughened by a few wart-like prominences; pale ochraceous salmon (R). No digestion or liquefaction of the medium.

Glycerin Potato (4 days). Good growth; raised; rough; dry; buff pink (R). (2 weeks) Becoming spreading; raised; surface roughened by a number of smooth wart-like prominences; edge erose; apricot buff (R).

Gelatin Stab. (7 days). Grayish-white, dry, rough, raised surface growth. Very scanty growth along line of inoculation.

III. Biochemical Features.

Litmus and Purple Milk. (4 days). Grayish pellicle. Alkaline. Slight coagulation in 2 weeks.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose or Sucrose.

Acid formed in 1% Glycerin Broth after 14 days.

Final Reaction in 5% Glycerin Broth.(4 weeks) pH 5.2.

Nitrate Broth and Agar. Nitrates not reduced to nitrites. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brown surface growth. No growth in the butt. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature for Growth.(on Dextrose Agar).

Optimum temperature for growth 30°C.

Maximum temperature for growth 37°C.

Minimum temperature for growth 8°C.

Relation to Reaction of the Medium.(5% Glycerin Broth).

Optimum H-ion conc. for growth; about pH 6.8 - 7.6.

Limits of pH for growth; from 5.0 to 10. 8

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

28. Mycobacterium rubrum.

I. Morphology. (Studied from B.P. Agar cultures at 35°C.).

Short rods, almost as thick as long, some cells almost spherical; majority of cells, like the yeast cell, ellipsoidal; 0.3 to 1.0 μ long and about the same width in the centre of the cell, tapering off to form a rounded end. Majority of cells about 0.5 μ in length. No longer or branched forms were observed. The culture has the same appearance after 1 month as it has after 24 hours of growth. The cells lie grouped together in pairs, like diplococci, in short chains, which is the most common grouping, and in irregular packets. Non-motile. No spores formed.

Staining Reactions. Age apparently has no effect on the staining characteristics, the organisms staining the same in cultures, 24 hours and 30 days old.

Gram-Stain. (Kopeloff and Beerman). Distinctly Gram-positive, the cells staining an intense blue. (Pl. 20, fig. 5.).

(Hucker). Gram-positive. The organisms do not stain as intensely, nor do they appear quite as large as by the Kopeloff and Beerman technique. (Pl. 20, fig. 6.).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol-fast. (Pl. 24, fig. 7.).

(Gabbet). Non-acid-fast. (Pl. 24, fig. 8.).

Muir's Capsule Stain. (2 months on Glycerin Agar). No capsules could be demonstrated.

II. Cultural Characters. (at 35°C.).

Gelatin colonies. (10 days).

Surface colonies. Very slow development. Colonies very small, about 0.5 m.m. in diameter; capitate; some appearing smooth, some matte; edge entire; red; internal structure, floccose. Not liquefied.

Deep colonies. Similar.

Dextrose Agar colonies (7 days).

Surface colonies. About 1.0 to 2.0 m.m. in diameter; irregular; raised; convex; surface is sometimes divided off into segments by definite ridges; edge lobate and filamentous; light coral red with a pale fringe; internal structure, floccose.

Deep colonies. Small, very irregular; lobate edge; filamentous (under low magnification).

Glycerin Agar Stroke. (48 hours). Good growth; spreading; flat; dry; wrinkled; edge undulate; coral red (R). (2 weeks) distinct paler arborescent fringe. Easily emulsified.

Dextrose Agar Stroke. (48 hours). Good growth, better on Dextrose than on Glycerin; spreading; flat; dry; membranous; slightly wrinkled; edge undulate; coral red (R). (2 weeks) Distinct paler, almost transparent arborescent fringe. (Pl. 28, fig. 2. B).

Nutrient Agar Stroke. Growth not as abundant as on Dextrose or Glycerin Agar; spreading; flat; thin; slightly wrinkled; edge lobate; flesh-pink in the deeper portions of growth, very pale at the border.

Dorset's Egg Medium. (2 weeks). Filiform; raised; dry; surface rough with a few warty prominences; light coral red (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Clear, with pink pellicle, which is deeply wrinkled, firm, very adherent and climbs up the sides of the flask. Slight pinkish sediment. (Pl. 31, fig. 2. C).

In some flasks if a surface film does not form, the organism grows as little fluffy balls on the bottom of the flask.

Loeffler's Blood Serum. (48 hours). Filiform, slightly moist pale pink growth, becoming in 7 days, spreading, dry, slightly roughened, rufus red (R). No digestion or liquefaction of the medium.

Glycerin Potato. (48 hours). Good growth; dull; dry; not wrinkled; nopal red (R).

Gelatin Stab. Red surface growth. Transparent, pinkish filmy growth along line of inoculation. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Red pellicle. Becoming alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose or Sucrose.

Acid formed in 1% Glycerin Broth after 7 days.

Final Reactions in 5% Glycerin Broth. (4 weeks) pH 5.6.

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Deep red surface growth. No growth in the butt. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature for Growth. (on Dextrose Agar).

Optimum temperature for growth	35°C.
Maximum temperature for growth	40°C.
Minimum temperature for growth	8°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion for growth; about pH 6.8 - 7.6.

Limits of pH for growth; from 5.5 - 10.5.

Indol Agar. Indigotin not formed

Blood Agar Plate. No haemolysis.

29. Mycobacterium salmonicolor.

I. Morphology. (Studied from B.P. Agar cultures at 35°C.).

Rods, varying in size from 3.0 to 27.0 μ in length by 0.5 to 0.75 μ in width; majority of bacilli about 3.0 to 6.0 μ long by about 0.5 μ wide. These rods are curved and of an even thickness throughout their length. Occasional branching is sometimes observed. Besides these rod forms, however, there are a large number of "coccoid" cells about 1.0 to 1.5 μ in size present in young cultures, 24 hours old, and increasing in number with age. In cultures, 7 days old, there are very few long rod forms present, while after 14 days, the "coccoid" forms are almost the only forms observed. These "coccoid" bacilli lie grouped together in short chains, giving the appearance sometimes of streptococci. Non-motile. No spores formed.

Staining Reactions.

Gram-Stain. (kopeloff and Beerman). (24 hours). The long rods and coccoid forms stain very intensely Gram-positive. (7 days). A large number of weakly staining, almost Gram-negative rods appear. (14 days) The number of these weakly staining cells increases, and the deeply staining cells decrease in number. (Pl. 26, figs. 1 and 2).

(Hucker). Gram-positive. The bacilli do not stain as intensely as by the Kopeloff and Beerman modification. A few cells stain very weakly. After 14 days the cells stain positively by Gram as in cultures, 24 hours old. (Pl. 26, figs. 3 and 4).

Acid-Fast Stain. (Ziehl-Neelsen). Not acid-alcohol fast. The organisms stain as blue granules in a pale blue, hardly visible, cell. (Pl 26, figs. 5 and 6).

(Gabbet). In cultures, 24 hours old, the bacilli are non-acid-fast, staining as by the Ziehl-Neelsen technique. As the culture gets older, however, a number of acid-fast granules or bacilli appear among the more numerous non-acid-fast organisms.(Pl.26, figs. 7 & 8).

Muir's Capsule Stain. (2 months on Glycerin Agar). The bacilli stain very unevenly, sometimes distinctly bipolarly. A fairly wide capsule is sometimes observed.

II. Cultural Characters. (at 35°C.).

Gelatin colonies. (10 days).

Surface colonies. Smaller colonies; about 0.7 m.m. in diameter; round; larger colonies rosulate, about 1.2 m.m. in diameter; pulvinate; varying from smooth to matte; edge lobate; pink; internal

structure, floccose, with a few arborescent projections from the edges. Not liquefied.

Deep colonies. Very much smaller, round, about 0.4 m.m. in diameter.

Dextrose Agar colonies (7 days).

Surface colonies. Large; from 1 to 3 m.m. in diameter; irregularly round; raised; surface broken into segments of different patterns, sometimes in rosette formation; edge definitely lobate; capucine buff (R); internal structure filamentous.

Deep colonies. Round; about 1 m.m. in diameter; yellow; edge very filamentous under low mangification.

Glycerin Agar Stroke. (48 hours). Moderate growth; isolated colonies, round, slightly raised, with segmented lobate edges and hollow depressions in the culture. When more numerous, coalescing to form a slightly spreading, slightly moist, rough, irregularly deeply lobed, coral red (R), streak. Easily emulsified.

Dextrose Agar Stroke. (48 hours). Very good growth; more rapid, thicker, and abundant than on Glycerin agar; spreading; very slightly moist; wrinkled; edge lobate and leaf-like fringed with very fine arborescent projections; orange buff (R).

(Pl. 28, fig. 2. A).

Nutrient Agar Stroke. (48 hours). Good growth; spreading; very slightly raised, almost flat; dry; membranous; wrinkled; lobate edge; ochraceous salmon (R). In older cultures, distinct very pale pinkish arborescent projections fringe the edge of the culture.

Dorset's Egg Medium. (2 weeks). Good growth; filiform; raised; moist; smooth; bullate; coral red (R). Medium not darkened or digested.

Glycerin Broth. (3 days in flasks). Pale pink, wrinkled pellicle, which is not very adherent to the flask but is easily broken into large flakes, which settle through the clear liquid slowly to the bottom of the flask.(Pl. 33, fig. 2. D).

Loeffler's Blood Serum. (48 hours). Good growth; filiform; raised; moist; smooth; orange, becoming in 2 weeks zinc orange and dry. No digestion or liquefaction of the medium.

Glycerin Potato. Good growth in 48 hours. (2 weeks) Becoming spreading; raised; granulated due to the dense, compact, fine wrinkles; edge irregularly cleft; orange (R).

Gelatin Stab. Orange surface growth. Very scanty, filmy growth along line of inoculation. No liquefaction.

III. Biochemical Features.

Litmus and Purple Milk. Pink pellicle. Alkaline. No coagulation or peptonization.

Carbohydrate Broths. No acid or gas produced from Dextrose, Lactose or Sucrose.

Acid formed in 1% Glycerin Broth after 6 weeks.

Final Reactions in 5% Glycerin Broth. (4 weeks) pH 6.2.

Nitrate Broth and Agar. Nitrates strongly reduced to nitrites in 24 hours. No gas formed.

Peptone Solution. Indol not formed.

Starch Agar. Starch not hydrolyzed.

Lead Acetate Agar. Brown surface growth. No growth in the butt. No blackening of the medium.

IV. Physiology.

Aerobic.

Relation to Temperature for Growth. (on Dextrose Agar)

Optimum temperature for growth	35°C.
Maximum temperature for growth	44°C.
Minimum temperature for growth	10°C.

Relation to Reaction of the Medium. (5% Glycerin Broth).

Optimum H-ion for growth; about pH 7.1 - 7.6.

Limits of pH for growth; from 5.0 to 11.0

Indol Agar. Indigotin not formed.

Blood Agar Plate. No haemolysis.

DISCUSSION OF RESULTS.

Morphology and Staining Reactions:

The Ziehl-Neelsen and Gabbet staining procedures divide the organisms studied roughly into four groups as follows:-

Group I. Acid-alcohol-fast.

No. 1. A and B. Mycobacterium tuberculosis hominis
2. Mycobacterium tuberculosis bovis
3. Mycobacterium paratuberculosis.
4. Mycobacterium avium.
6. Mycobacterium marinum.
7. Mycobacterium ranae.
9. Mycobacterium chelonei.
10. Mycobacterium smegmatis.
11. Mycobacterium butyricum.
12. Mycobacterium berolinensis
13. Mycobacterium stercusis
14. Mycobacterium phlei
15. Mycobacterium graminis.

Group II. Acid-fast, but not acid-alcohol-fast.

No. 8. Mycobacterium thamnopheos.

Group III. Not acid-alcohol-fast; doubtfully acid-fast.

No.	16.	Mycobacterium actinomorphum.
	17.	Mycobacterium agreste.
	18.	Mycobacterium album.
	20.	Mycobacterium convolutum.
	21.	Mycobacterium crystallophagum.
	25. A and B.	Mycobacterium lacticola
	27.	Mycobacterium opacum
	29.	Mycobacterium salmonicolor

Group IV. Not acid-fast.

No.	5.	* Mycobacterium piscium.
	19.	Mycobacterium coeliacum.
	22.	Mycobacterium erythropolis.
	23.	Mycobacterium globerulum.
	24.	† Mycobacterium hyalinum.
	26.	Mycobacterium luteum.
	28.	Mycobacterium rubrum.

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* The strain of Mycobacterium piscium studied does not conform to the descriptions of the organism given by Bataillon, Dubard and Terre (5), who first isolated it, or by Griffith and others, who have since studied it. It is described by previous investigators as a pleomorphic, Gram-positive, acid-fast bacillus, very similar in its morphology and staining properties to the tubercle bacillus of human tuberculosis. The strain studied was found to be very different; it was a very short rod, similar in appearance to Escherichia coli, Gram-negative and non-acid-fast. It produced turbidity in glycerin broth and the cultural characters differed somewhat from the findings of previous investigatos. From these results, then, this organism does not belong in the genus Mycobacterium and will not be further considered in this discussion.

The staining difference between the third and fourth groups is too indefinite a character to separate their members into two different genera. The greater number of organisms in Group III show acid-fast rods or coccoid bacilli, only in older cultures, The young, typical rod-shaped form is not acid-fast.

Group II, represented by one species, Mycobacterium thamnopheos is distinctly acid-fast but it is decolorized by the alcohol treatment. This organism, however, differs from the members of Group III, in that the acid-fastness is a more constant and definite character.

Group I contains the acid-alcohol-fast bacilli, typified by the true tubercle bacillus and its closely related types.

For convenience, however, these organisms can better be divided into two groups:-

Group I. Acid-fast bacilli, including the members of Groups I and II above.

Group II. Not-acid-fast or doubtfully acid-fast bacilli, including Groups III and IV above.

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† Mycobacterium hyalinum differed considerably from the other organisms studied. It produced a thin, smooth, moist, at first almost transparent, greenish growth on the culture media used, very different from the rough, wrinkled, characteristic growth of the group. Furthermore, it differed greatly in its morphology; it was a very short, slender rod, not acid-fast or Gram-positive, not forming coccoid rods, beaded cells, or filamentous or branched forms. These results show this organism to be so very different from the organisms in both groups that it should not be considered as being in the same genus or genera as these organisms.

The morphology of these organisms seems also to divide them roughly into two groups. The most common, typical shape of the young bacillus in this genus is a short, slender, slightly curved rod. This was found to be the case with the members of Group I. They occurred most commonly as short, slender, curved rods, and only the occasional, longer, thread-like form was observed at all. Although filamentous and branched forms have been reported as a common character of this group, these forms seem only to be present in any appreciable number among the true tubercle bacilli types, when conditions of environment are somewhat adverse for proper development. In Group II, however, the young forms of the greater number of the organisms were frequently very long, highly curved, sometimes branched rods, which, as the culture aged, split into short coccoid cells, similar to the fragmentation spores of the Actinomyces.

The staining reactions were somewhat affected by the age of the culture in the case of some of the organisms studied. All the species of Group I were acid-fast in young and old cultures, but this property was more marked in the older cultures of some organisms. In cultures, 24 hours old, there were often a number of non-acid-fast staining rods, whereas, after 2 weeks growth, hardly any of these forms could be found. Amongst the members of

Group II, all the species were distinctly non-acid-fast in young cultures, but in the older cultures of some of the organisms, granules or portions of a cell sometimes exhibited this property.

The size of the greater number of the organisms studied was materially affected by the age of the cultures. The bacilli were generally longer and thicker in cultures, 24 hours old, than in cultures, 48 or 72 hours old. Although this phenomenon was quite pronounced among the members of Group I, it was most marked in cultures of the soil forms isolated by Gray and Thornton and by den Dooren de Jong.

The differences in the size of the bacilli of Group II are too small and indefinite to offer any basis for "species" differentiation, which is in agreement with the findings of the great majority of investigators.

None of the organisms studied was motile; this is contrary to the findings of Ferran (25) and Schumowski (78).

No spores were observed in any of the cultures.

Granular and beaded forms were very common; the Kopeloff and Beerman modification of the Gram stain brought out the granules more clearly than the other staining procedures.

A large number of the organisms were embedded in a mass of gummy-like, capsular material, but it was very

difficult to demonstrate capsules around the individual cells, except in the case of Mycobacterium globerulum and Mycobacterium lacticola, in which the capsules were very marked.

Cultural Reactions:

A few marked differences were observed between species. Mycobacterium rubrum and Mycobacterium agreste could be differentiated from the other members of the group by their characteristic deep red pigmentation on the culture media used. Amongst the members of Group I, much significance could not be attached to pigment formation as a specific differentiating character. Almost all the organisms of this group produced some pigment, which generally varied from shades of buff to red.

The general character of the growth on artificial media was raised, somewhat rough or wrinkled, and gradually spreading. Some forms, however, such as Mycobacterium globerulum and Mycobacterium lacticola, formed a thick, soft, smooth, slimy growth.

Most of the organisms grew equally well on Glycerin and Dextrose agar. Glycerin seemed somewhat more favorable in the cultivation of Mycobacterium tuberculosis hominis (I b.); Mycobacterium avium and Mycobacterium paratuberculosis, while Dextrose gave better results with the soil Mycobacteria.

All the organisms that grew well in glycerin broth,

with the exception of Mycobacterium marinum, produced pellicles in flasks of this medium. The nature of this pellicle varied from a thin, fragile, easily broken membrane to a thick, crumpled, tough, adherent growth. The liquid generally remained clear during the first few days of growth, but soon became turbid due to the settling of flakes broken from the pellicle.

All the organisms, with the exception of Mycobacterium paratuberculosis, grew on Loeffler's Blood Serum, Dorset's Egg Medium and Glycerinated Potato, and pigment production was especially good on these media in many instances. None of the organisms caused any discoloration or digestion of these media.

Gelatin was not liquified by any of the species.

Biochemical Reactions:

None of the organisms produced acid in dextrose, sucrose or lactose.

A rather striking difference was observed between the members of Groups I and II in the final reaction produced by them in 5% glycerin broth. All the organisms of Group II, with the exception of Mycobacterium luteum, produced an acidity in glycerin broth above pH 6.0, the majority of cultures giving a reaction of between pH 5.2 and 5.6. Whereas, all the broth cultures of organisms in Group I, were more alkaline than pH 6.0, the greater number remaining around neutrality. Although the

organisms of Group II produced an acidity in 5% glycerin broth as indicated by a determination of the pH, only Mycobacterium globerulum, Mycobacterium agreste, Mycobacterium rubrum, Mycobacterium opacum, Mycobacterium salmonicolor and Mycobacterium hyalinum formed any acid in fermentation tubes containing 1% glycerin broth with 1% Andrade's indicator. The differentiation observed by Theo. Smith between the human and bovine types by their reactions in glycerin broth was not observed.

None of the organisms studied hydrolyzed starch. None produced indol from peptone, which is not in agreement with the results of Vierling, Sohngen, Gray and Thornton, Rabinowitsch, Petri, and of Korn, mentioned previously.

The reduction of nitrates to nitrites by 16 of the species studied was very marked, and this character was not limited to the organisms of any particular group.

Milk was rendered alkaline by most of the organisms. Mycobacterium marinum alone produced a slight acidity.

Physiological Reactions.

On Indol agar, only Mycobacterium globerulum produced indigotin.

All the organisms of both groups grew well on the blood agar plate, but none had any effect on the erythrocytes, with the exception of Mycobacterium

crystallophagum, which, at first, produced a small haemolytic zone around the area of growth, and subsequently caused a greening of the plate.

The production of Hydrogen sulphide was a very indefinite character. As none of the organisms grew in the stabbed lead acetate agar, no blackening of the medium was observed. They, however, grew well on the surface of the agar, and the growth, in many instances, gradually assumed a brown to blackish coloration.

With regard to the effect of the reaction of the medium on growth, this study showed that almost all the organisms possessed a wide pH range for growth. It was observed that almost all the non-acid-fast types, the organisms of Group II, grew on a somewhat more alkaline medium than the acid-fast species. The greater number of them grew in a medium of pH 10.8 - 11.0, whereas few of the acid-fast types grew in a medium more alkaline than pH 10.5. No growth was observed in any of the cultures in media more alkaline than pH 11.5. The limit of growth on the acid side for the majority of organisms was about pH 5.0; a few species, however, grew at pH 4.0.

In this study, an interesting observation was made in regard to the adjustment of the reaction of alkaline media. Sterilized melted glycerin agar, at 40°-44°C., when adjusted to pH 11.5, with sterile NaOH by means of indicators, and not subjected to any further treatment,

showed a rapid and luxuriant growth of every organism studied. But few of these organisms, however, grew in a glycerin broth, of reaction pH 11.0, while most of them grew but scantily in a medium of pH 10.0 - 10.5. It seems very evident, then, that some reaction takes place in the agar medium in which the alkali is quickly made more neutral. The reverse phenomenon was not observed in the acid media.

The organisms with few exceptions, possessed a rather wide temperature range for growth. They may be divided into groups on the basis of their temperature requirements. (1). Organisms growing well at 37°C. and temperatures slightly higher. In this group, fall most of the acid-fast bacilli, with the exception of some of the strains of tubercle bacilli isolated from cold-blooded animals, and the non -acid-fast saprophytes, Mycobacterium rubrum and Mycobacterium agreste. Some of these organisms can grow at a very high temperature; most of them grow at 52°C. and Mycobacterium phlei grew at 58°C. (2). Organisms not growing above 37°C. but growing well between 20° and 30°C. In this group, fall the non-acid-fast saprophytes of Group II.

A number of these organisms grow at temperatures as low as 8° - 10°C.

Mycobacterium avium, Mycobacterium paratuberculosis and Mycobacterium tuberculosis hominis (1 B) posses a

very narrow temperature range for growth. The virulent human tubercle bacillus and Mycobacterium paratuberculosis do not grow below 36°C. and above 44°C. and Mycobacterium avium does not grow below 32°C. and above 44°C.

The Systematic Relationships of the Organisms Studied.

As mentioned in the historical discussion earlier in this paper, as soon as acid-fast organisms have been discovered they have been classified with the other bacilli characterized by this property. Question has not unnaturally been raised as to the wisdom of forming a genus solely on the basis of this single staining property. Long (95), however, is of the opinion that acid-fastness is something more than a mere staining peculiarity. He considers this reaction as indicating some common chemical component of these acid-fast bacilli, which separates them from other bacterial groups, and accepts the findings of Cooke (19) and others on the antigenic properties of acid-fast organisms, stating that there is evidence that the acid-fastness is associated with a certain antigenic value in common, which can be demonstrated in a qualitative way. "The impression remains" he concludes "based upon the possession of acid-fastness and similar antigenic value, that a phylogenetic relationship does exist."

This possession of a common antigenic property by the acid-fast bacilli has been much contested, and the evidence for such a statement is anything but conclusive. It is considered by Calmette (14) and many others that there is no common antigenic value for the acid-fast group of bacteria.

In the case of some organisms, acid-fastness seems to be a very definite character. Just as certain organisms are Gram-variable, so certain organisms may be acid-fast variable. When, however, acid-fastness is very definite, and is associated with a number of characteristic cultural resemblanees, it seems legitimate to use this staining property as a basis of generic differentiation. It might be mentioned here that there are a number of acid-fast organisms that have not been classified in the genus Mycobacterium, for example, the acid-fast Actinomycetes, so that acid-fastness is not used solely as a basis of classification, but only when in association with other definite, cultural and physiological characters.

Accepting the definition of the genus Mycobacterium as proposed by Lehmann and Neumann and employed by Bergey, only the organisms of Group I, the true acid-fast organisms, should be classified in this genus. The organisms in Group II, on account of their non-acid-fastness, their other morphological differences, such as the definite splitting of the young filamentous-like rods into

cocci, similar to the fragmentation spores of the Actinomyces, and their other slight cultural and physiological differences, should be separated into another genus.

Whether their similarities to the members of the genus Actinomyces justifies their classification with the Actinomycetes, or whether they should be placed with the Corynebacteria, or in a new genus is a problem yet to be solved by the systematists, and no classification is attempted in this paper. It should be mentioned, however, that all the organisms of Group II, with the exceptions stated, seem to form a very definite group, and are not to be further subdivided. Bergey places 5 of the six Mycobacteria, described by Gray and Thornton, in the genus Actinomyces and the sixth in the genus Flavobacterium; he also classifies Mycobacterium salmonicolor as Flavobacterium salmonicolor. There is no justification for such a separation, since on a basis of pigmentation alone more than two of the species could be transferred to that genus.

Differentiation of Species in Group I, the Genus Mycobacterium.

Mycobacterium paratuberculosis may, first, be separated from the other members of this group, by its paucity of growth on culture media and the difficulty of cultivating it at all.

Temperature relationships make a sharp division in this group. (1). Those bacilli not growing at 37°C., having an optimum temperature for growth at 20° - 25°C. i. e., Mycobacterium chelonei, Mycobacterium marinum and Mycobacterium thamnopheos.

Mycobacterium chelonei and Mycobacterium marinum are acid-alcohol-fast. Mycobacterium thamnopheos is not. Mycobacterium chelonei produces little pigment, its growth being generally grayish-white, Mycobacterium marinum forms a deeply pigmented yellow growth on the culture media used. (2). Those micro-organisms growing best at 37°C. i.e., Mycobacterium tuberculosis hominis (both strains), Mycobacterium bovis, Mycobacterium avium, Mycobacterium ranae, Mycobacterium smegmatis, Mycobacterium butyricum, Mycobacterium berolinensis, Mycobacterium stercusis, Mycobacterium phlei, Mycobacterium graminis.

This investigation showed a number of differences between the saprophytic and the virulent strains of Mycobacterium tuberculosis hominis. Morphologically, they were alike, but they differed culturally in the extent and rapidity of their growth, the virulent strain growing very slowly, the saprophytic strain growing rapidly and luxuriantly; the saprophytic strain was also more deeply pigmented. The most striking differences, however, were observed in their temperature and pH relationships. The saprophytic strain had a very wide temperature range

for growth 15° - 52°C., while the virulent strain grew only between 36°&44°C. The pH range for growth of the saprophytic culture was 4.4 to 10.0, while for the virulent strain it was 5.0 to 9.0.

The virulent, freshly-isolated bovine tubercle bacillus is characterized by its very slow, poor development on culture media, and this character has been used by many to differentiate it from the tubercle bacillus of human origin. In this investigation, a saprophytic strain of Mycobacterium tuberculosis bovis is described which grows well on the ordinary culture media, being indistinguishable from the saprophytic human bacillus.

Mycobacterium avium can be distinguished from (1) the virulent strain of Mycobacterium tuberculosis hominis by the consistency of growth on glycerin agar; it is rather smooth, soft and soapy-like, while the growth of Mycobacterium tuberculosis hominis is rough, wrinkled and very coherent, and (2), from the remainder, by its slow growth on the culture media used.

Amongst the other members of this group, there are no sharply defined differentiating characters. Mycobacterium butyricum is generally much shorter and thicker, almost coccus-shaped and its growth on culture media is generally smoother and a brighter yellow than the others.

The others are closely alike. Mycobacterium tuberculosis hominis (saprophytic strain), Mycobacterium tuberculosis bovis and Mycobacterium ranae cannot be distin-

guished morphologically or culturally. Mycobacterium smegmatis, Mycobacterium stercusis, Mycobacterium phlei, Mycobacterium graminis and Mycobacterium berolinensis posses no sharp differences but the appearance of their growth on culture media show slight specific differences. Mycobacterium phlei can grow at a somewhat higher temperature - 58°C. and Mycobacterium berolinensis and Mycobacterium stercusis produce a little lower final reaction in glycerin broth.

SUMMARY AND CONCLUSIONS.

1. The morphology and physiology of thirty-one organisms classified as Mycobacteria have been described.

2. By means of the Ziehl-Neelsen and Gabbet staining procedures, these organisms have been divided into two groups, (1) Acid-fast, and (2), Non-acid-fast bacilli.

3. Although these two groups posses a large number of characteristics in common, the definition of the genus Mycobacterium as given by Lehmann and Neumann, has been accepted, and, accordingly, only the acid-fast organisms are considered as belonging to this genus.

4. The following organisms are classified in the genus Mycobacterium: Mycobacterium tuberculosis hominis (two strains); Mycobacterium tuberculosis bovis; Mycobacterium paratuberculosis; Mycobacterium avium; Mycobacterium ranae; Mycobacterium chelonei; Mycobacterium marinum; Mycobacterium smegmatis; Mycobacterium butyricum; Mycobacterium berolinensis; Mycobacterium stercusis; Mycobacterium phlei; Mycobacterium graminis and Mycobacterium thamnopheos.

5. In this genus, the saprophytic strains of the human and bovine tubercle bacilli and Mycobacterium ranae are indistinguishable. Temperature relationships, rapidity and consistency of growth, pigmentation and cultural characters on the media employed, serve to differentiate the other species.

6. Studies were made of the non-acid-fast bacilli

and their characters compared with those of the acid-fast species.

7. The non-acid-fast organisms, with the exception of Mycobacterium piscium and Mycobacterium hyalinum, which are Gram-negative and unlike the other organisms studied, form a homogeneous group, which possesses many characters in common with certain members of the genus Actinomyces. In old cultures, some of these organisms occasionally show some acid-fast cells. It is not, however, proposed to establish the systematic position of this group of organisms.

8. Filamentous and branched forms in very young culture, with the subsequent division of these cells into cocci, similar to the fragmentation spores of the Actinomyces, is a common characteristic of the non-acid-fast group. Granular and beaded forms are observed amongst the acid-fast bacilli, but no definite separation of the young vegetative cell into these oidia-like bodies was observed. Filamentous and branched forms were only occasionally observed amongst the acid-fast types, and these generally in old cultures.

9. In both groups, the organisms are all non-motile, Gram-positive (with the exceptions stated above), and a number produce a gummy, capsular-like material.

10. All the species produce pigment, which varies from a pale green to a deep red.

11. The organisms of the non-acid-fast group possess a number of cultural characters, which are similar to those of the acid-fast types.

12. In 5% glycerin broth, the non-acid-fast species produce a more alkaline reaction than the acid-fast.

13. With the exception of a few virulent acid-fast strains, the organisms had a wide range for growth in relation to temperature and reaction of the medium, the majority of the non-acid-fast strains being able to grow in a somewhat more alkaline medium.

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TABLE 1.

DISTINCTIVE BIOCHEMICAL CHARACTERS

- of Group 1. -

PHYSIOLOGICAL CHARACTERS

- of Group 1. -

	Culture Number	Litmus & B.C.P. Milk	Carbohydrate Broths (1 %) Dextrose Sucrose Lactose	Carbohydrate Broths (1 %) Glycerin	Reaction in 5 % Glycerin (4 weeks) p.h.	Nitrates Reduced to Nitrites	Relation to Temperature -°C.- Min.	Opt.	Max.	Relation to p.h. of Medium Min.	Opt.	Max.	Indigotin produced from Indol.	Haemolysis on Blood Agar Plate
M.tuberculosis hominis	1.A.	Alk.	-	-	6.8	++	15°	37°	52°	4.4	6.6-7.3	10.0	-	-
	1.B.	Alk.	-	-	6.8	-	35°	37°	44°	5.0	6.8-7.6	9.5	-	-
M.tuberculosis bovis	2.	Alk.	-	-	7.0	++	15°	37°	52°	4.0	6.8-7.3	10.0	-	-
M.paratuberculosis	3.	No growth					-	40°	-	-	-	-	- No growth	
M.avium	4.	No change	-	-	7.2	-	32°	40°	44°	5.0	6.8-7.3	8.5	-	-
M.piscium	5.	Alk.	-	-	6.2	-	10°	20°-25°	32°	5.0	6.8	10.0	-	-
M.marinum	6.	Acid	-	-	7.0	-	10°	20°-25°	37°	5.8	6.8	10.8	-	-
M.ranae	7.	Alk. Peptonized	-	-	7.0	++	10°	37°	52°	4.0	6.6-7.3	10.0	-	-
M. thamnopheos	8.	Alk.	-	-	7.0	-	10°	25°	35°	5.0	7.3-8.0	11.0	-	-
M.chelonei	9.	Alk.	-	-	7.0	-	18°	25°	32°	5.0	6.8-7.1	10.8	-	-
M.smegmatis	10.	Alk.	-	-	6.8	++	15°	37°	52°	4.4	6.6-7.8	10.8	-	-
M.butyricum	11.	Alk.	-	-	7.0	++	15°	35°	44°	5.5	6.6-7.1	10.5	-	-
M.berolinensis	12.	Alk.	-	-	6.4	++	20°	37°	52°	4.4	7.1-7.6	10.5	-	-
M.stercusis	13.	Alk.	-	-	6.4	++	15°	37°	52°	5.0	6.6-7.3	10.5	-	-
M.phlei	14.	Alk.	-	-	6.8	++	20°	37°	58°	5.5	6.8-7.3	10.5	-	-
M.graminis	15.	Alk.	-	-	7.0	++	18°	37°	52°	4.0	6.8-8.5	10.5	-	-

TABLE 2.

DISTINCTIVE BIOCHEMICAL CHARACTERS

PHYSIOLOGICAL CHARACTERS

- of Group 2.-

- of Group 2.-

	Culture Number	Litmus & B.C.P. Milk	Carbohydrate Broths (1%)		Reaction in 5% Glycerin (4 weeks) p.h.	Nitrates Reduced to Nitrites	Relation to Temperature -°C.-			Relation to p.h. of Medium			Indigotin produced from Indol.	Haemolysis on Blood Agar Plate
			Dextrose Sucrose Lactose	Glycerin			Min.	Opt.	Max.	Min.	Opt.	Max.		
M.actinomorphum	16.	Alk.	-	-	6.0	-	8°	25°-30°	37°	5.0	7.8-8.5	11.0	-	-
M.agreste	17.	Alk.	-	Acid in 14 days	5.4	++	8°	37°	48°	5.0	6.8-8.0	11.0	-	-
M.album	18.	Alk.	-	-	5.4	-	10°	25°-30°	35°	4.4	7.8-8.5	11.2	-	-
M.coeliacum	19.	Alk.	-	-	5.2	-	8°	25°	35°	5.0	7.1-7.3	11.2	-	-
M.convolutum	20.	Alk.	-	-	5.2	-	8°	25°-30°	37°	5.0	7.6-8.0	10.8	-	-
M.crystallophagum	21.	Alk.	-	-	6.0	+	8°	30°	37°	4.4	6.8-7.3	11.0	-	++
M.erythropolis	22.	Alk.	-	-	5.2	+	8°	25°	35°	5.0	6.8-8.0	11.2	-	-
M.globerulum	23.	Alk. Peptonized	-	Acid in 2 days	5.0	++	10°	28°	37°	5.0	6.8-7.6	11.0	++	-
M.hyalinum	24.	No Change	-	Acid in 7 days	5.2	-	8°	20°	35°	5.2	7.1-8.5	11.2	-	-
M.lacticola	25.	Alk.	-	-	5.2	++	8°	25°	37°	5.0	6.8-7.8	11.2	-	-
M.luteum	26.	Alk.	-	-	6.8	-	8°	25°	37°	5.0	6.6	11.0	-	-
M.opacum	27.	Alk. Litmus reduced	-	Acid in 14 days	5.2	-	8°	30°	37°	5.0	6.8-7.6	10.8	-	-
M.rubrum	28.	Alk.	-	Acid in 7 days	5.6	++	8°	35°	40°	5.5	6.8-7.6	10.5	-	-
M.salmonicolor	29.	Alk.	-	Acid in 30 days	6.2	++	10°	35°	44°	5.0	7.1-7.6	11.0	-	-

Note 1. Starch not hydrolyzed by any of the species.

2. Indol not formed from peptone by any of the species.

PLATE 1.

Mycobacterium tuberculosis hominis (1 A.).

(from B. P. Agar).

x 1200

Fig.1- 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman Gram Stain.

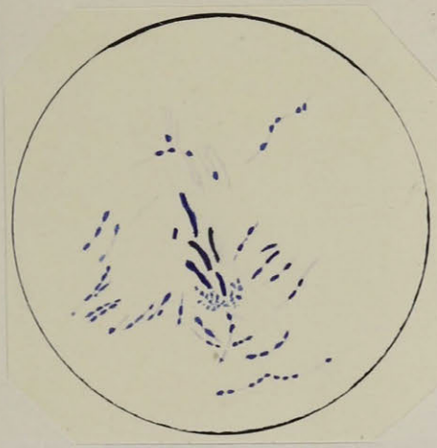
Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 14 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 14 days. Gabbet's Stain.

PLATE 1.

Fig. 1.



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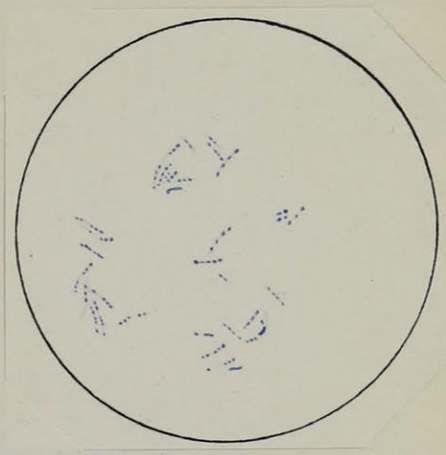

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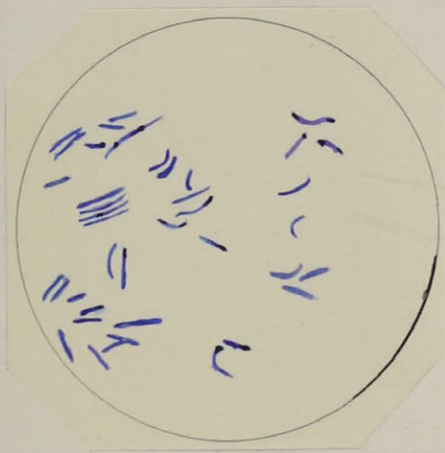
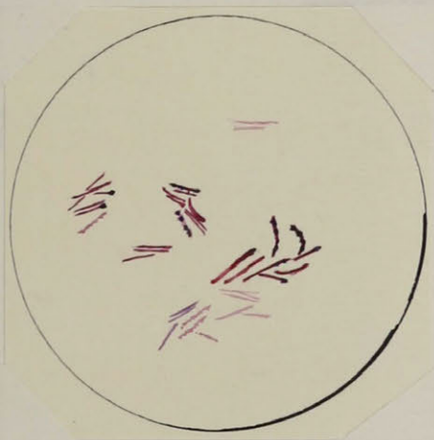


Fig. 4.

Fig. 5.



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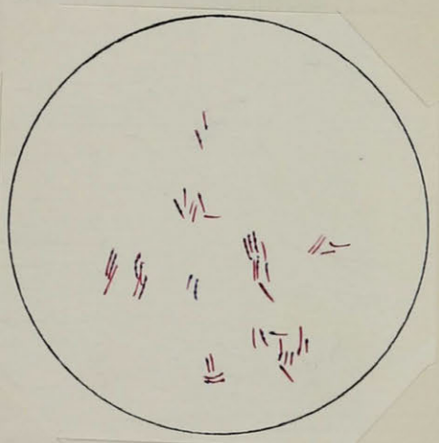

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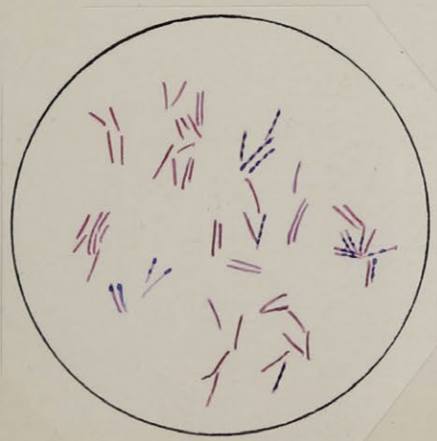

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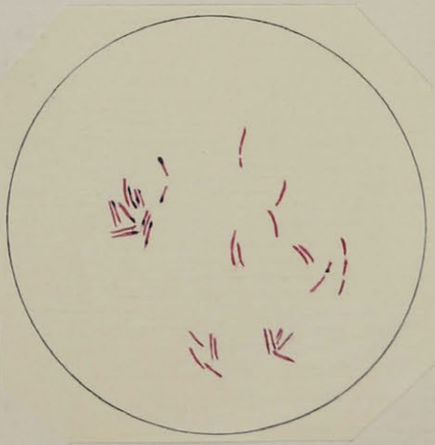


PLATE 2.

Mycobacterium tuberculosis hominis (1. B).

(from Glycerin Agar).

x 1200

Fig. 1. - 7 days. & Fig. 2. - 2 months. Kopeloff and Beerman Gram Stain.

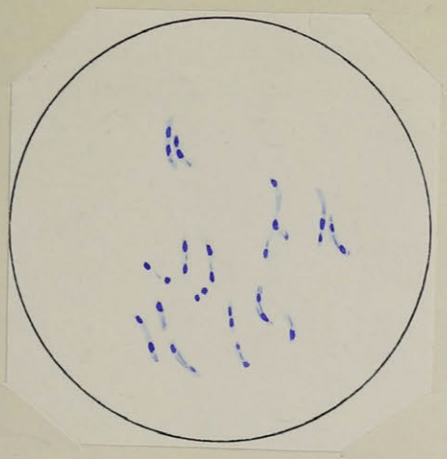
Fig. 3. - 7 days. & Fig. 4. - 2 months. Hucker's Gram Stain.

Fig. 5. - 7 days. & Fig. 6. - 2 months. Ziehl-Neelsen Stain.

Fig. 7. - 7 days. & Fig. 8. - 2 months. Gabbet's Stain.

PLATE 2.

Fig. 1.



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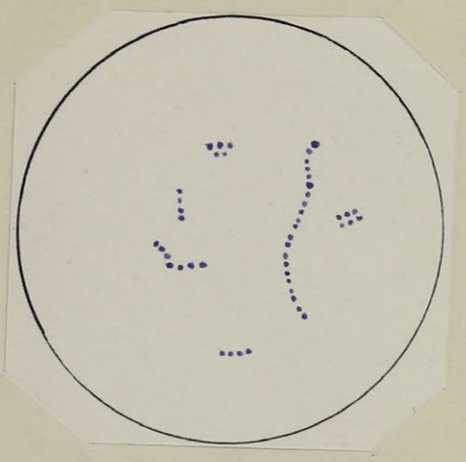


Fig. 3.

Fig. 4.

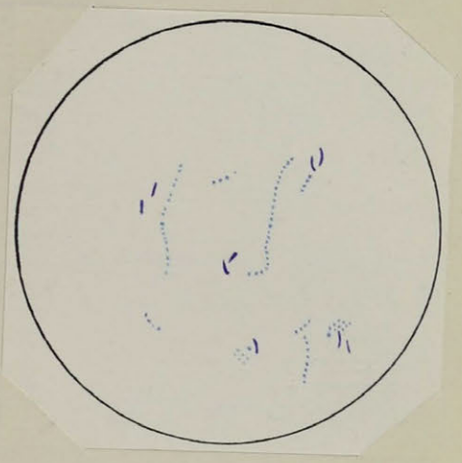
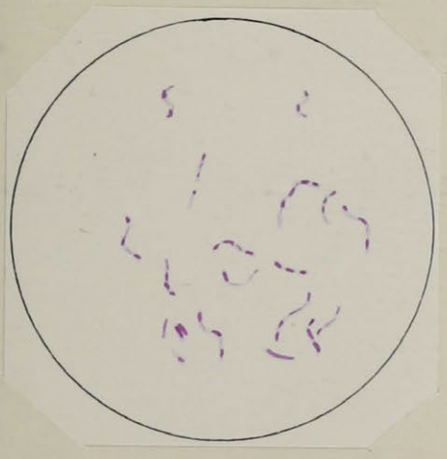


Fig. 5.



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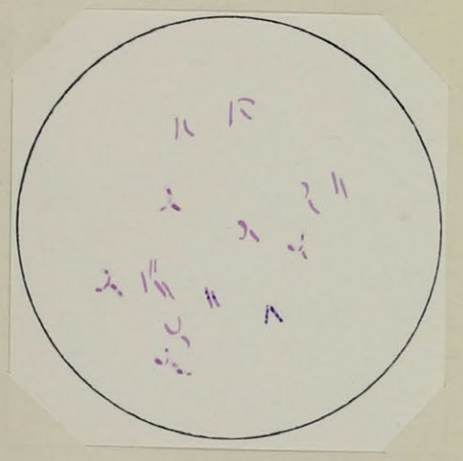


Fig. 7.

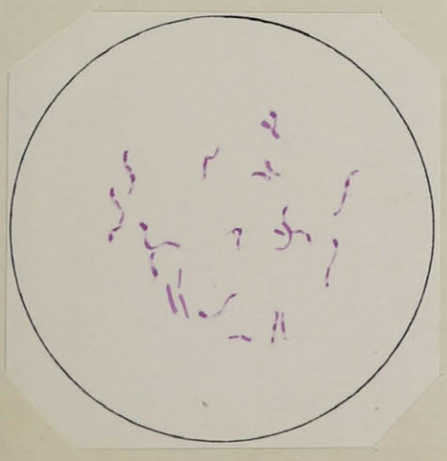


Fig. 8.

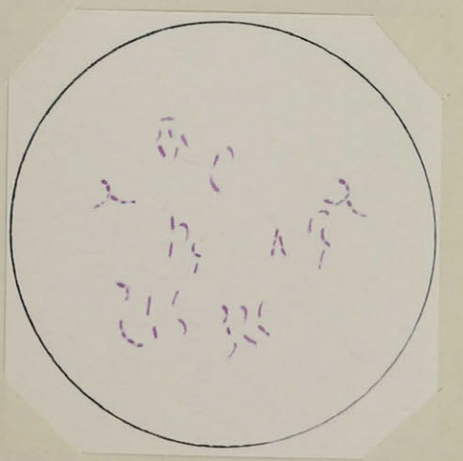


PLATE 3.

Mycobacterium tuberculosis bovis.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

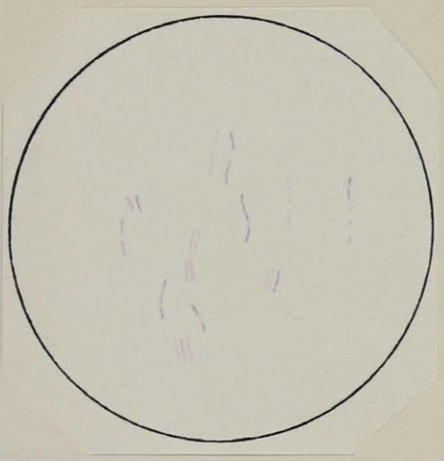
Fig. 5. - 1 day. & Fig. 6. - 14 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 14 days. Gabbet's Stain.

PLATE 3.

Fig. 1.

Fig. 2.



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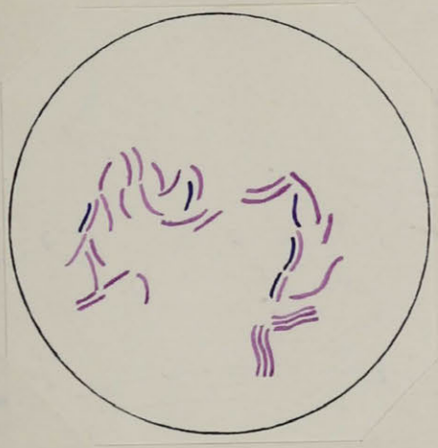

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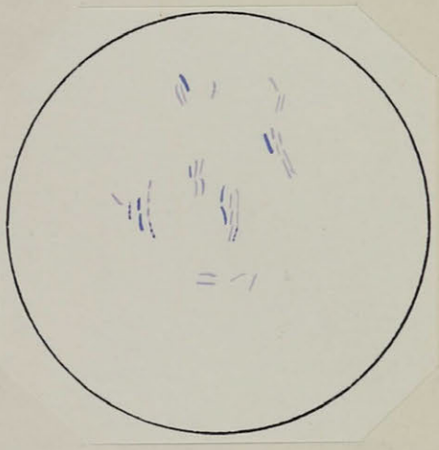
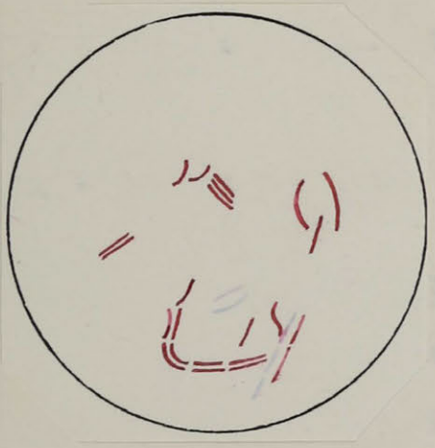


Fig. 5.



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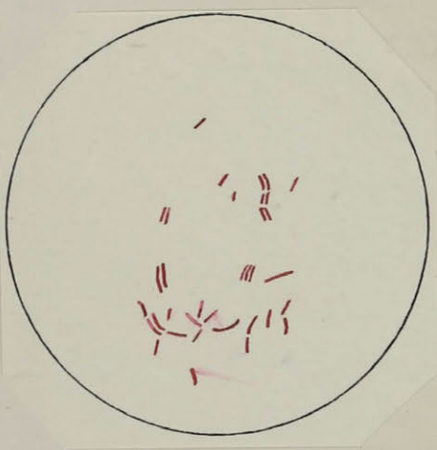


Fig. 7.

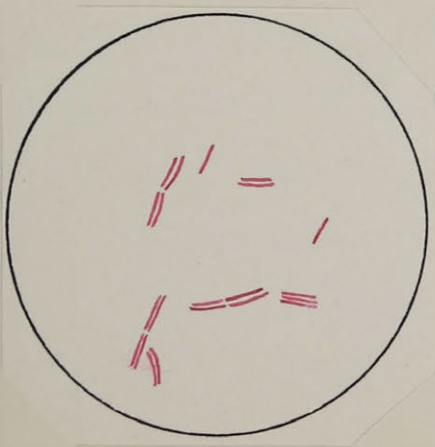


Fig. 8.

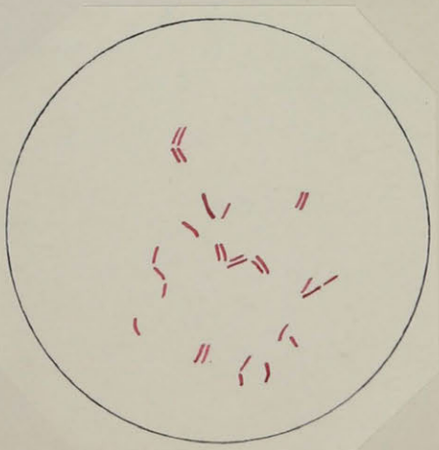


PLATE 4.

Mycobacterium paratuberculosis.

(from Glycerin Agar - 1 month).

x 1200.

Fig. 1. - Kopeloff and Beerman Gram Stain.

Fig. 2. - Hucker's Gram Stain.

Fig. 3. - Ziehl-Neelsen Stain.

Fig. 4. - Gabbet's Stain.

Mycobacterium avium.

(from Glycerin Agar - 14 days).

x 1200.

Fig. 5. - Kopeloff and Beerman Gram Stain.

Fig. 6. - Hucker's Gram Stain.

Fig. 7. - Ziehl-Neelsen Stain.

Fig. 8. - Gabbet's Stain.

PLATE 4.

Fig. 1.

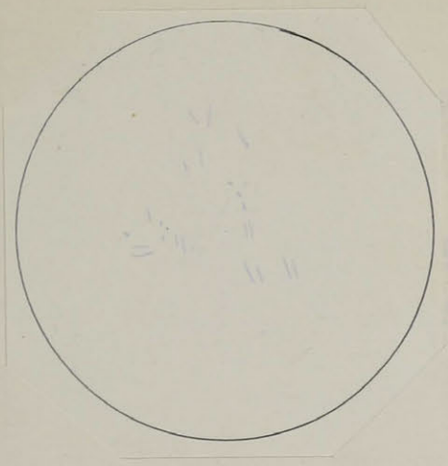


Fig. 2.

Fig. 3.

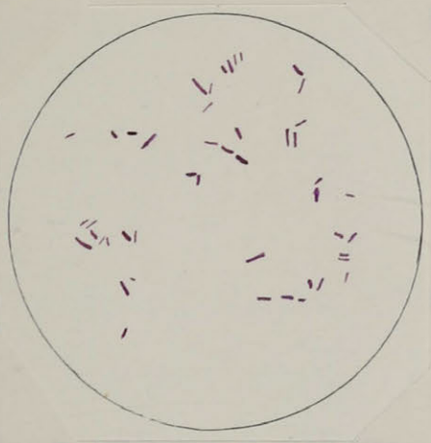
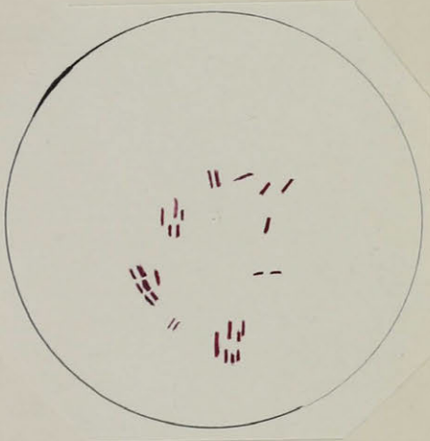


Fig. 4.



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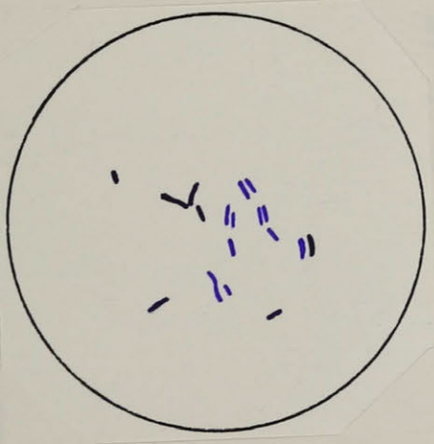


Fig. 6.

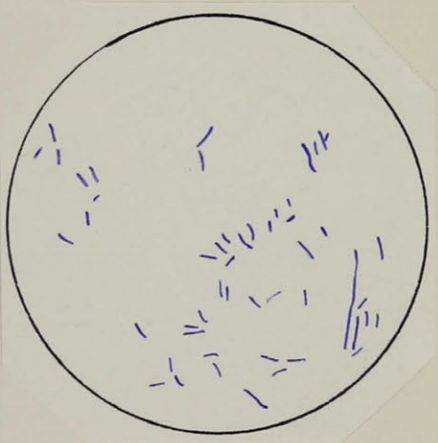


Fig. 7.

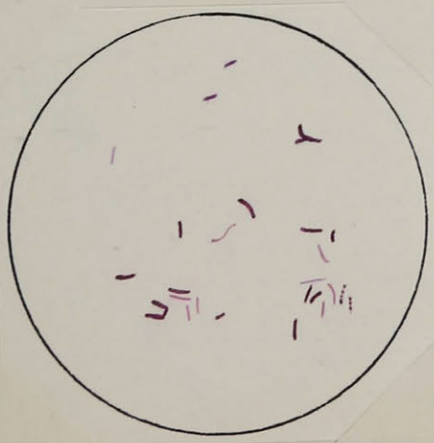


Fig. 8.

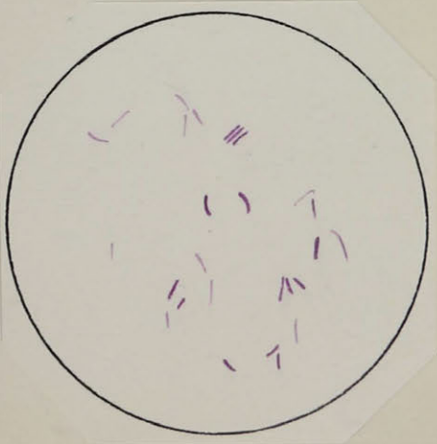


PLATE 5.

Mycobacterium piscium.

(from B. P. Agar - 1 day).

x 1200.

Fig. 1. - Köpeloff and Beerman Gram Stain.

Fig. 2. - Hucker's Gram Stain.

Fig. 3. - Ziehl-Neelsen Stain.

Fig. 4. - Gabbet's Stain.

Mycobacterium marinum.

(from B. P. Agar).

x 1200

Fig. 5. - 1 day & Fig. 6. - 4 weeks. Hucker's Gram Stain.

Fig. 7. - 1 day. - Ziehl-Neelsen Stain.

Fig. 8. - 1 day. - Gabbet's Stain.

PLATE 5.

Fig. 1.

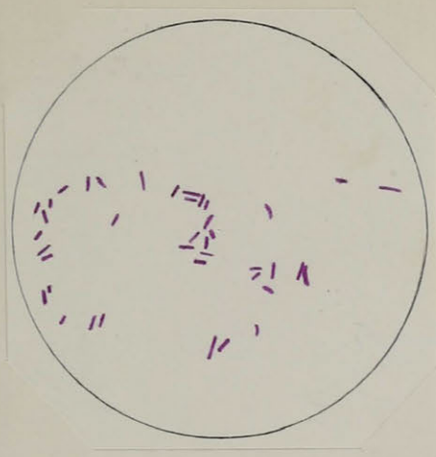


Fig. 2.

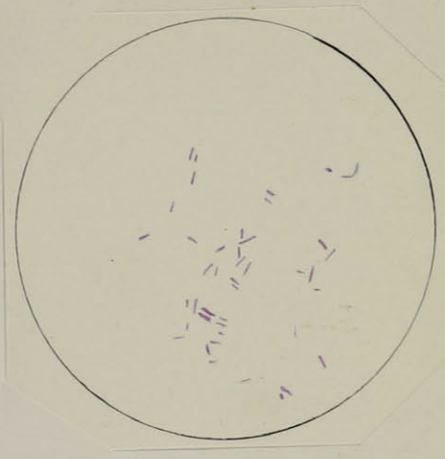


Fig. 3.

Fig. 4.

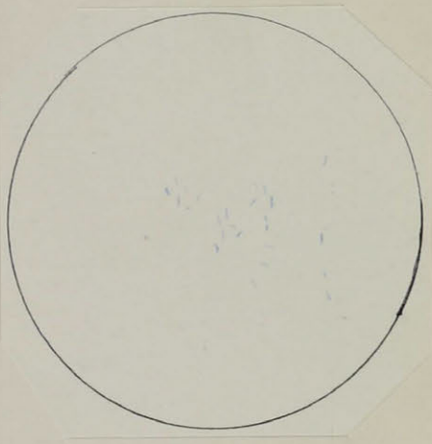


Fig. 5.

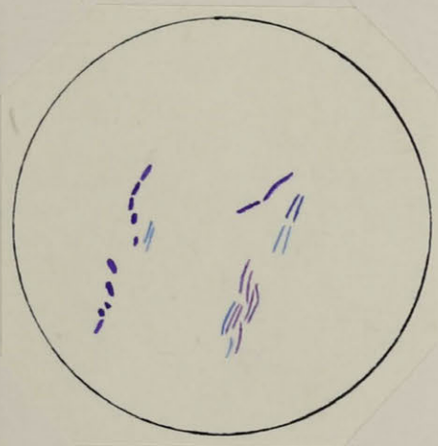


Fig. 6.

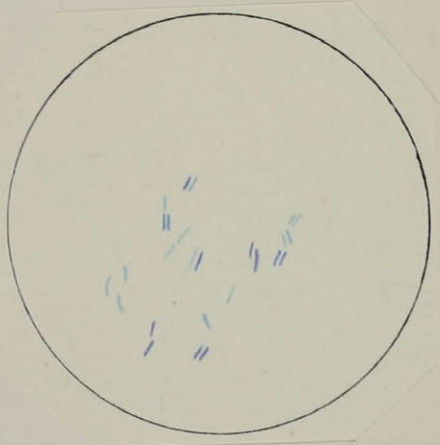


Fig. 7.

Fig. 8.

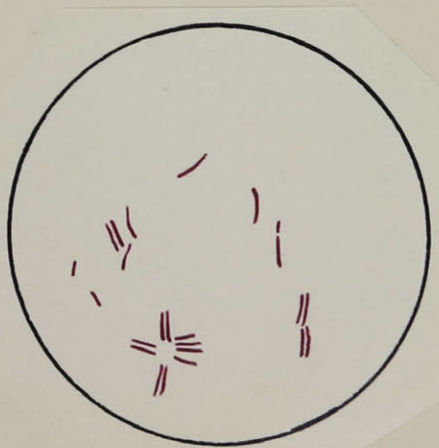


PLATE 6.

Mycobacterium ranae.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 14 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 14 days. Gabbet's Stain.

PLATE 6.

Fig. 1.

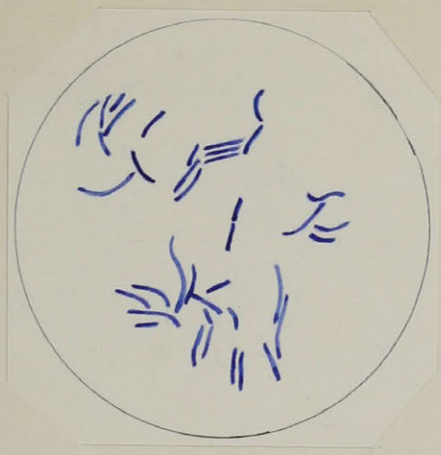


Fig. 2.

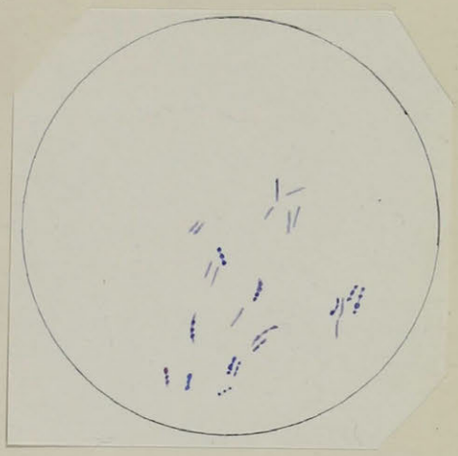


Fig. 3.

Fig. 4.

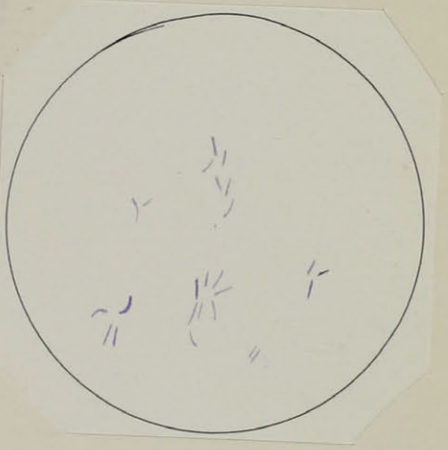


Fig. 5.

Fig. 6.

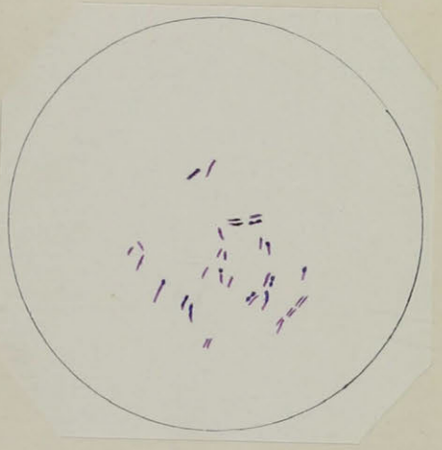


Fig. 7.

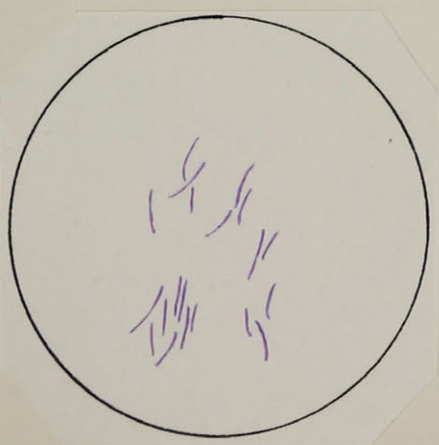


Fig. 8.

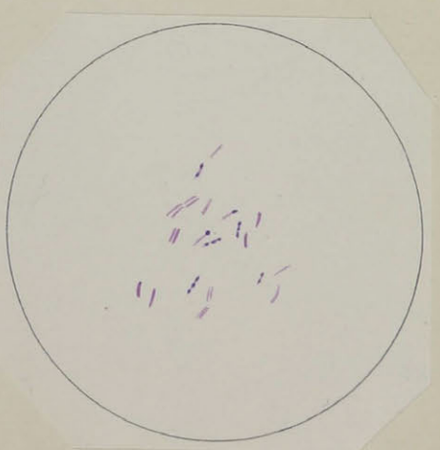


PLATE 7.

Mycobacterium thamnopheos.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 30 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 30 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 30 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 30 days. Gabbet's Stain.

PLATE 7.

Fig. 1.

Fig. 2.

Fig. 3.

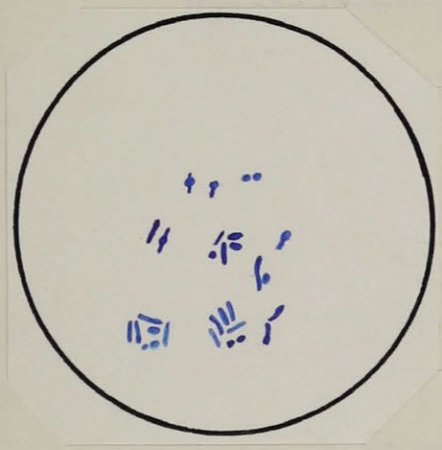


Fig. 4.

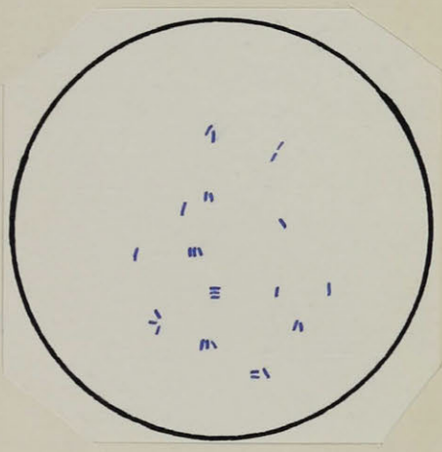


Fig. 5.

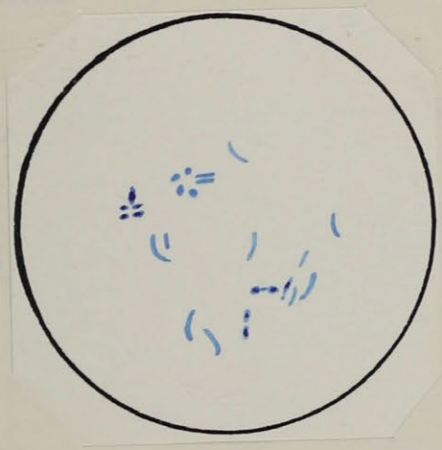


Fig. 6.

Fig. 7.

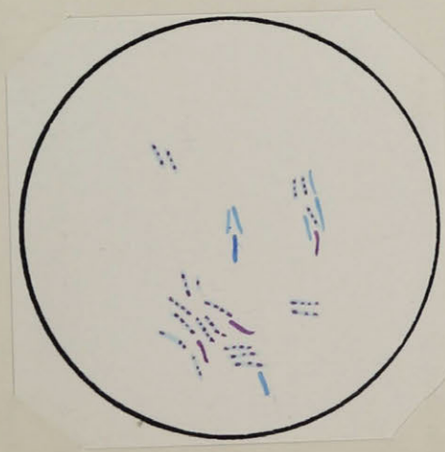


Fig. 8.

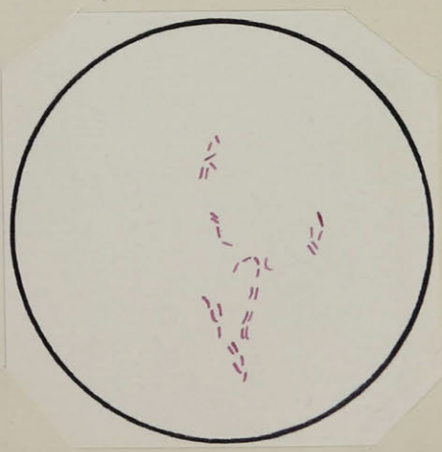


PLATE 8.

Mycobacterium chelonei.

(from B. P. Agar - 1 day).

x 1200.

Fig. 1. - Kopeloff and BeermanGram Stain.

Fig. 2. - Hucker's Gram Stain.

Fig. 3. - Ziehl-Neelsen Stain.

Fig. 4. - Gabbet's Stain.

PLATE 8.

Fig. 1.

Fig. 2.

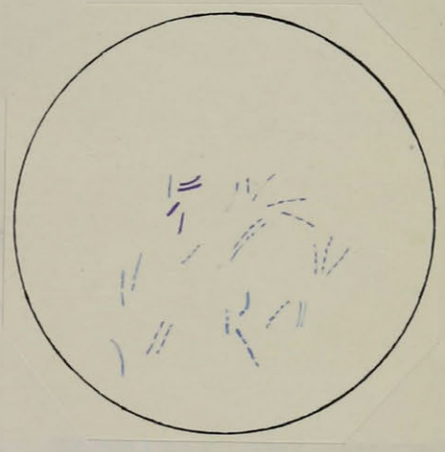


Fig. 3.

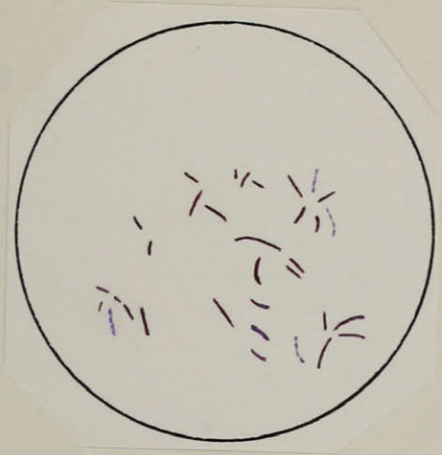


Fig. 4.

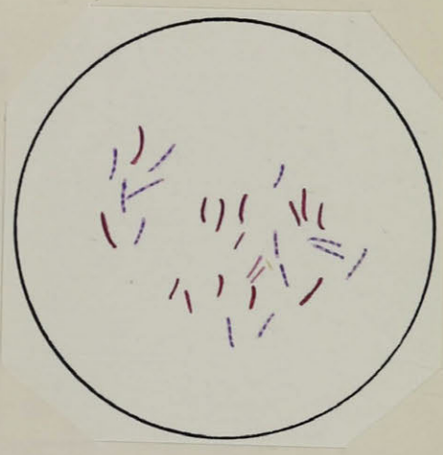


PLATE 9.

Mycobacterium smegmatis.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 30 days. Kopeloff and Beerman Gram Stain.

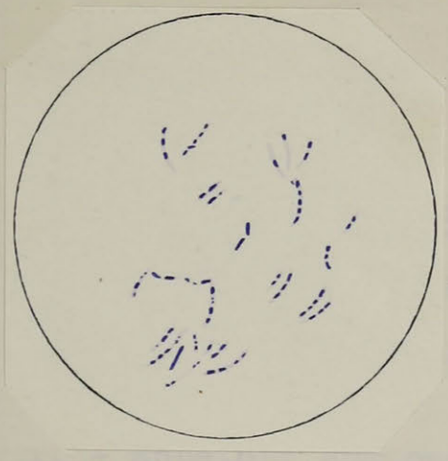
Fig. 3. - 1 day. & Fig. 4. - 30 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 30 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 30 days. Gabbet's Stain.

PLATE 9.

Fig. 1.



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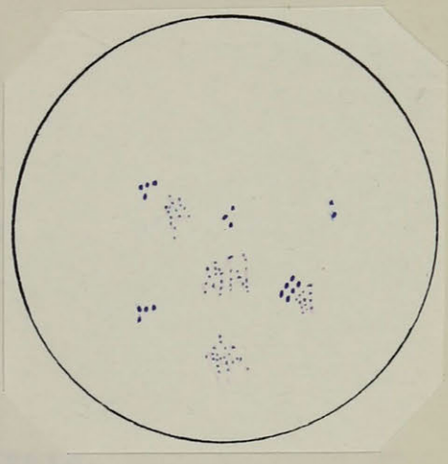


Fig. 3.

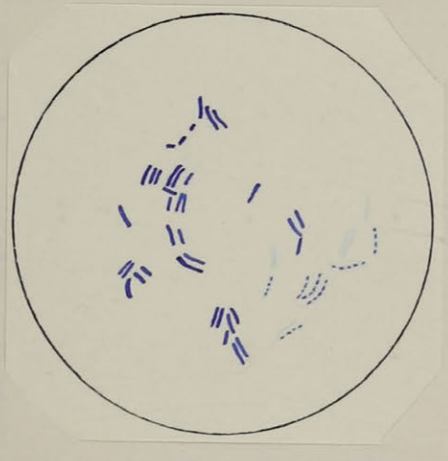


Fig. 4.

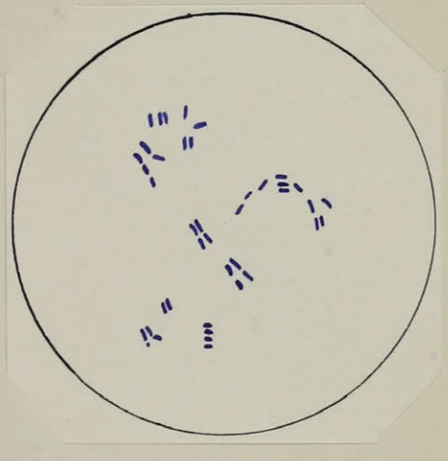


Fig. 5.

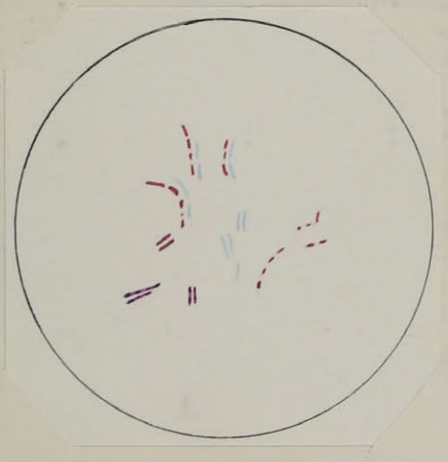


Fig. 6.

Fig. 7.

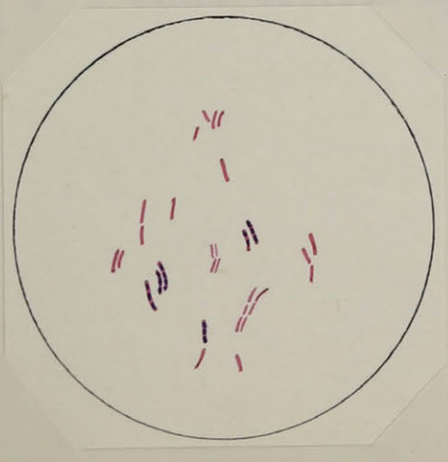


Fig. 8.

PLATE 10.

Mycobacterium butyricum.

(from B. P. Agar - 1 day).

x 1200.

Fig. 1. - Kopeloff and Beerman Gram Stain.

Fig. 2. - Hucker's Gram Stain.

Fig. 3. - Ziehl-Neelsen Stain.

Fig. 4. - Gabbet's Stain.

Mycobacterium berolinensis.

(from B. P. Agar - 1 day).

x 1200.

Fig. 1. - Kopeloff and Beerman Gram Stain.

Fig. 2. - Hucker's Gram Stain.

Fig. 3. - Ziehl-Neelsen Stain.

Fig. 4. - Gabbet's Stain.

PLATE 10.

Fig. 1.

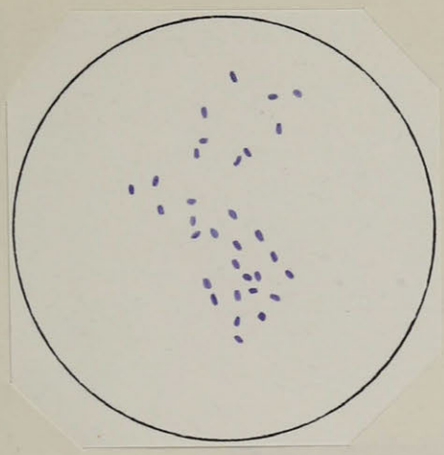


Fig. 2.

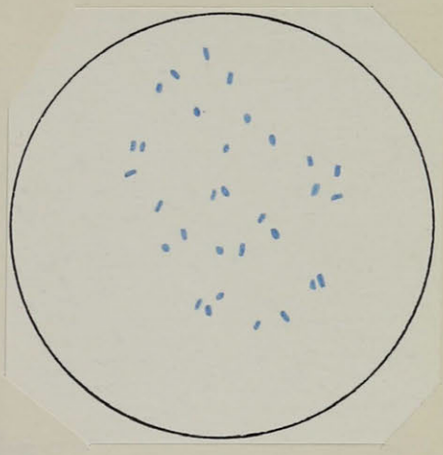


Fig. 3.

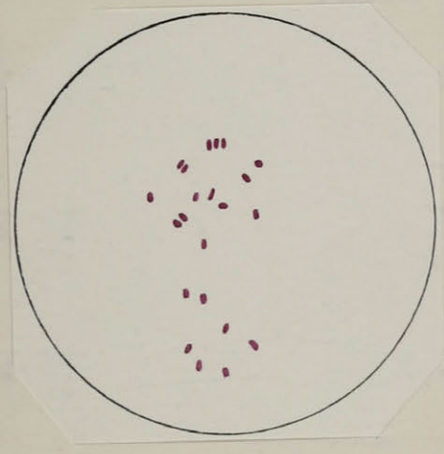


Fig. 4.

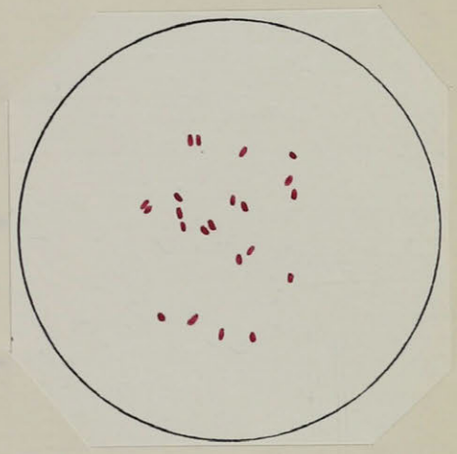


Fig. 5.

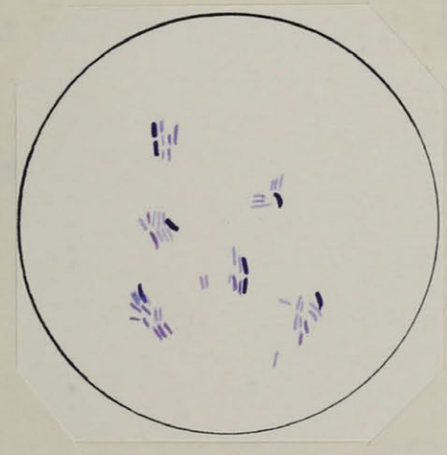


Fig. 6.

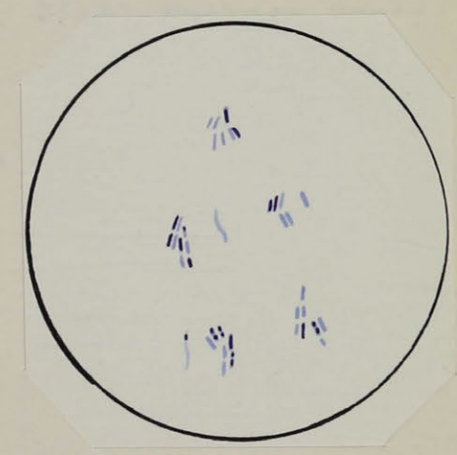


Fig. 7.

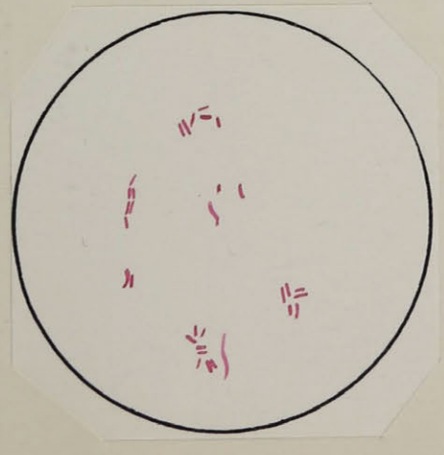


Fig. 8.

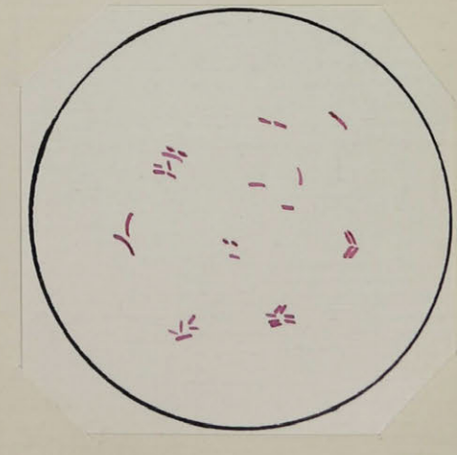


PLATE 11.

Mycobacterium stercusis.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 30 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 30 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 30 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 30 days. Gabbet's Stain.

PLATE 11.

Fig. 1.

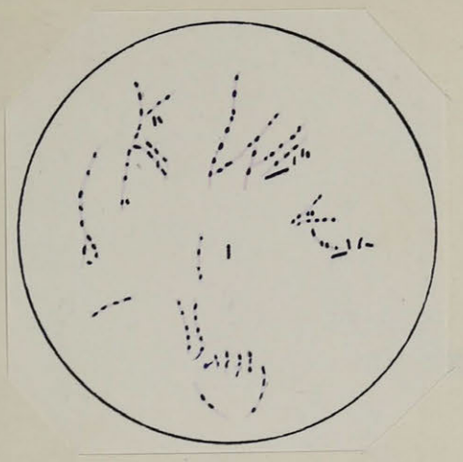


Fig. 2.

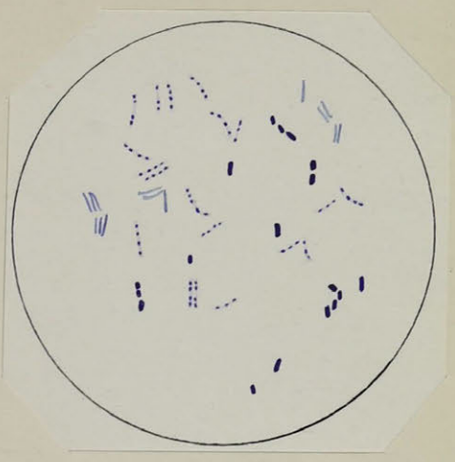


Fig. 3.

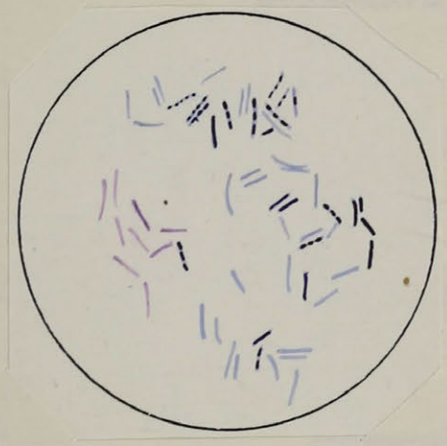


Fig. 4.

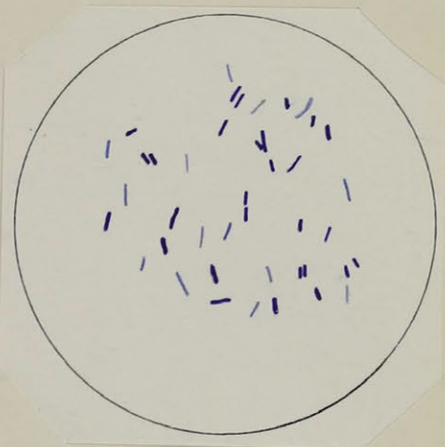


Fig. 5.

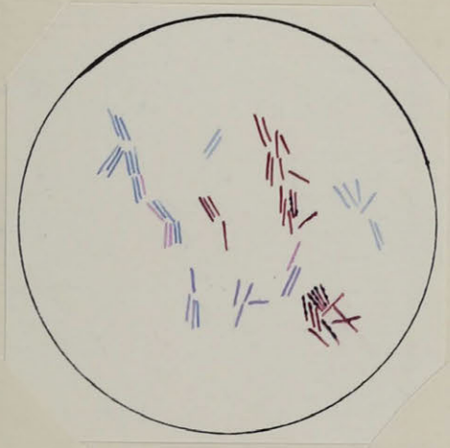


Fig. 6.

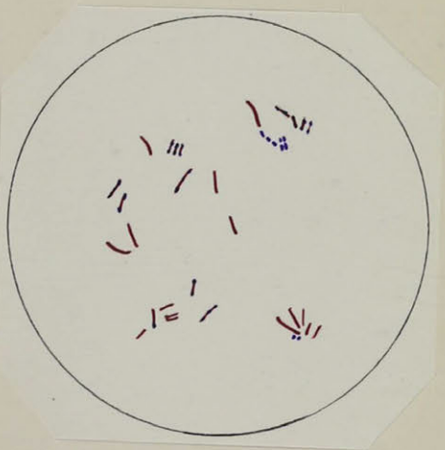


Fig. 7.

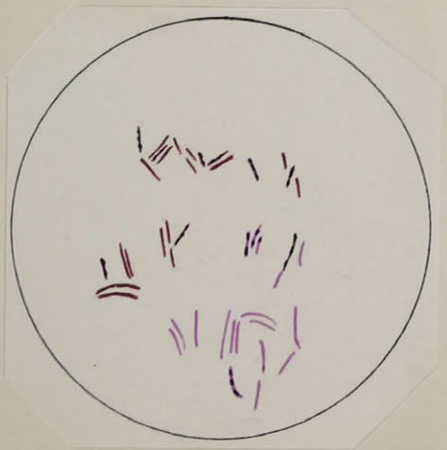


Fig. 8.

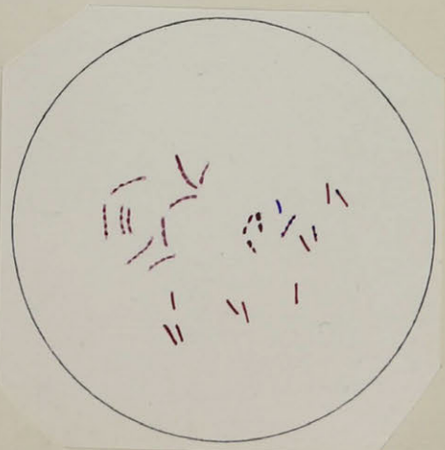


PLATE 12.

Mycobacterium phlei.

(From B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 14 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 14 days. Gabbet's Stain.

PLATE 12.

Fig. 1.

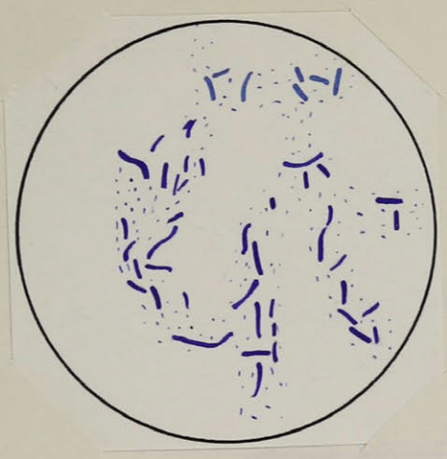


Fig. 2.

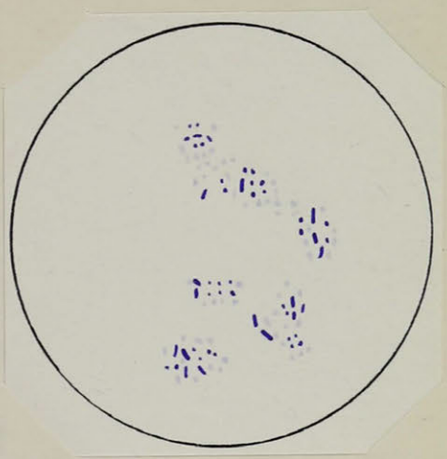


Fig. 3.

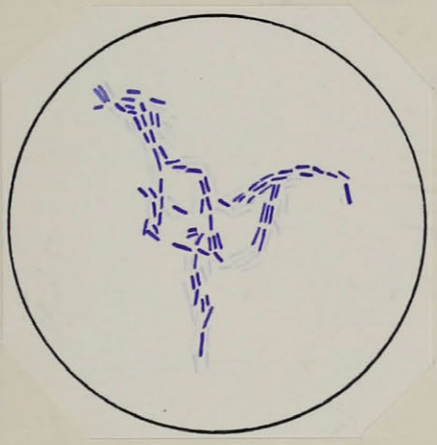


Fig. 4.

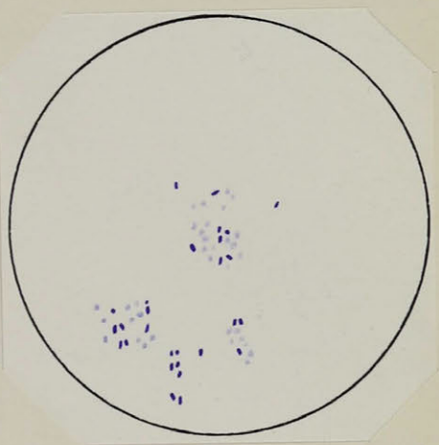


Fig. 5.

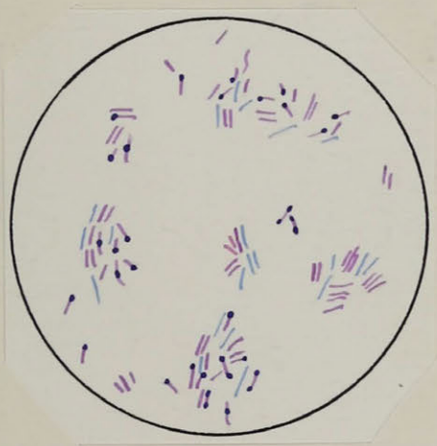


Fig. 6.

Fig. 7.

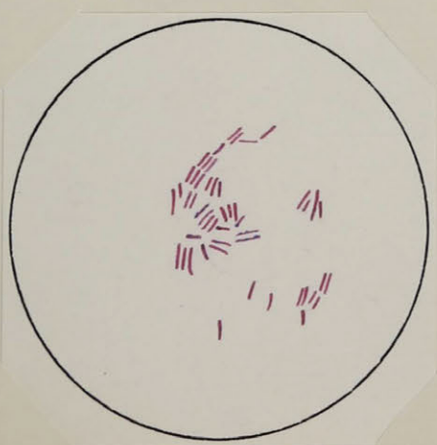


Fig. 8.

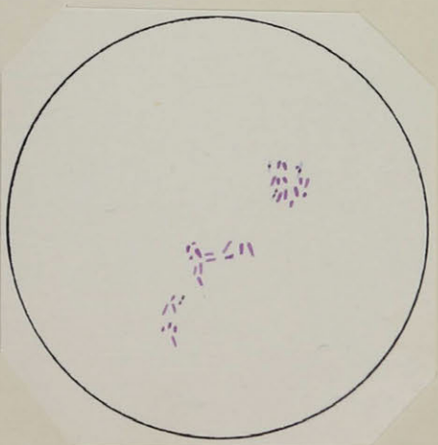


PLATE 13.

Mycobacterium graminis.

(from B. P. Agar),

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 14 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 14 days. Gabbet's Stain.

PLATE 13.

Fig. 1.

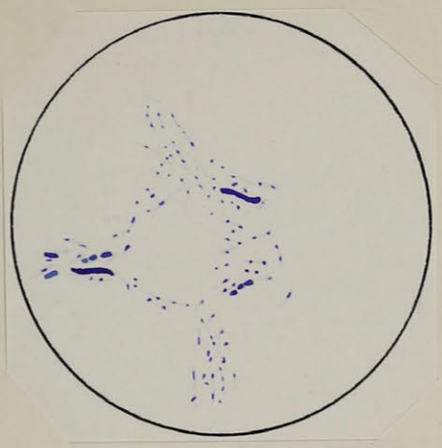


Fig. 2.

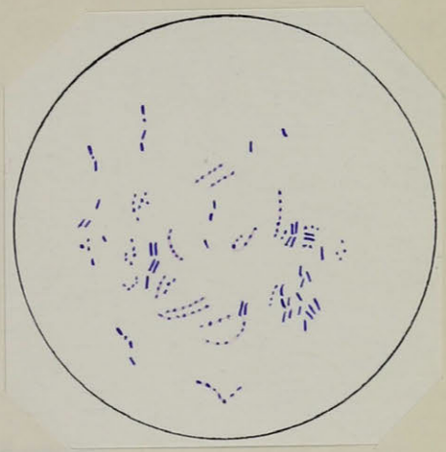


Fig. 3.

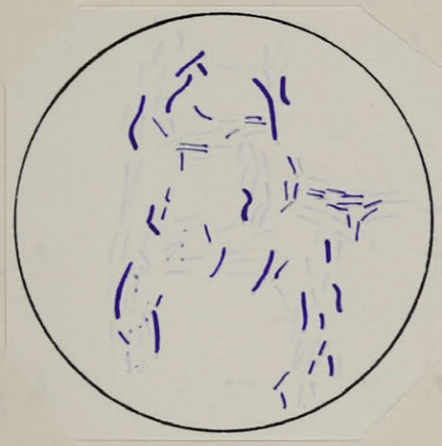


Fig. 4.

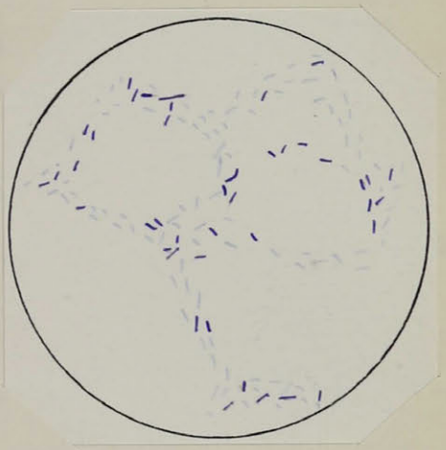


Fig. 5.

Fig. 6.

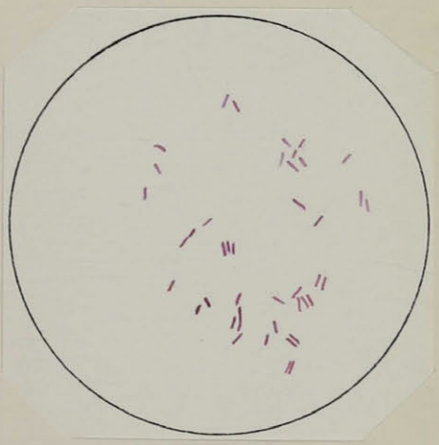


Fig. 7.

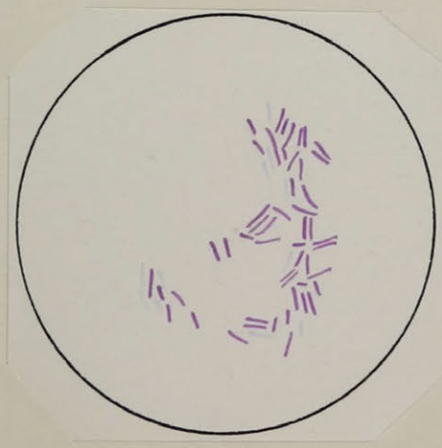


Fig. 8.

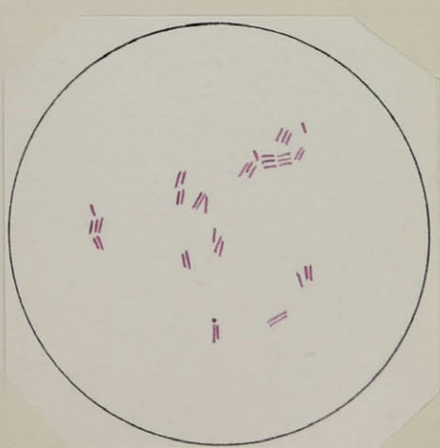


PLATE 14.

Mycobacterium actinomorphum.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 30 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 30 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 30 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 30 days. Gabbet's Stain.

PLATE 14.

Fig. 1.

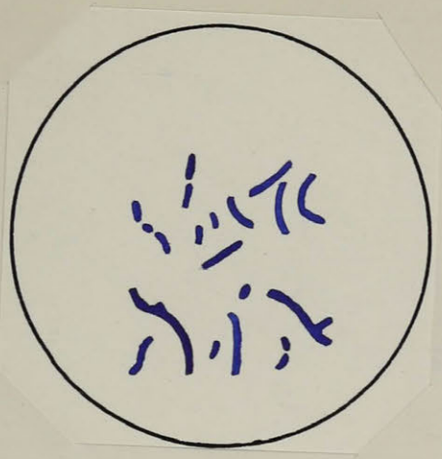


Fig. 2.

Fig. 3.

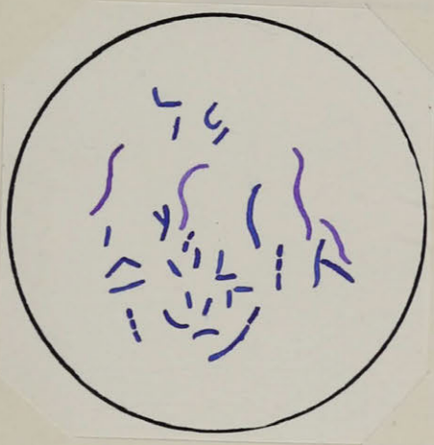


Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

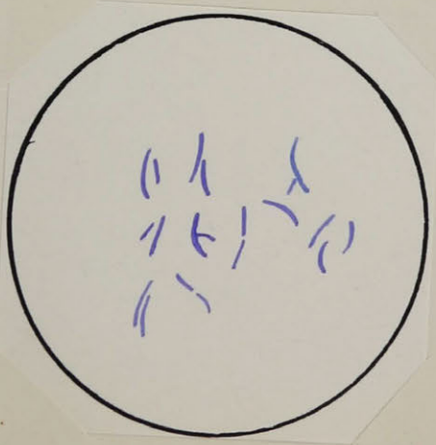


Fig. 8.

PLATE 15.

Mycobacterium agreste.

(from B.P. Agar).

x 1200.

Fig. 1 - 1 day. Kopeloff and Beerman Gram Stain.

Fig. 2. - 1 day. Hucker's Gram Stain.

Fig. 3. - 1 day. Ziehl-Neelsen Stain.

Fig. 4. - 1 day. & Fig. 5. - 30 days. - Gabbet's Stain.

PLATE 15.

Fig. 1.

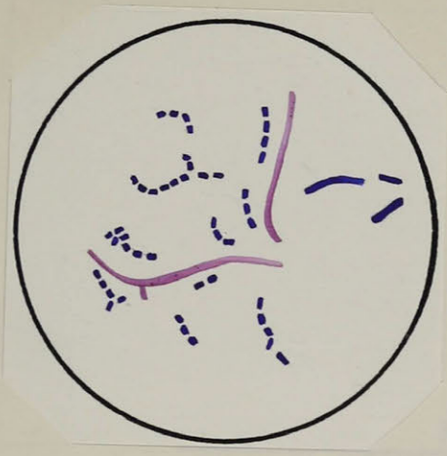


Fig. 2.

Fig. 3.

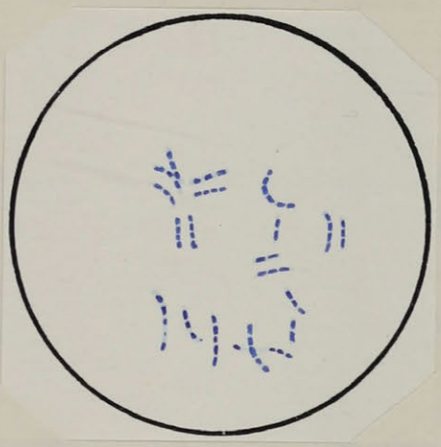


Fig. 4.

Fig. 5.

PLATE 16.

Mycobacterium album.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 14 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 14 days. Gabbet's Stain.

PLATE 16.

Fig. 1.

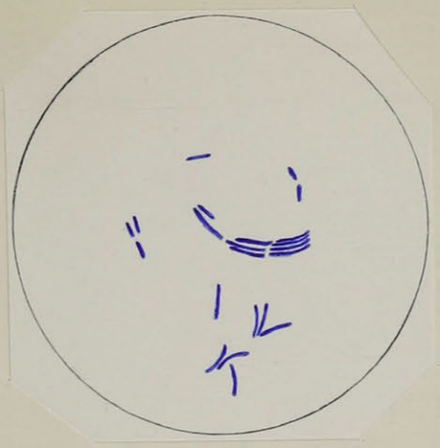


Fig. 2.

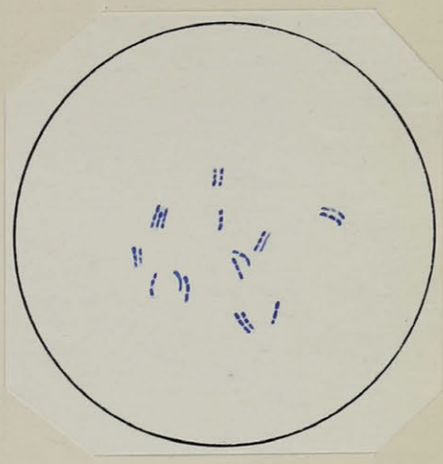


Fig. 3.

Fig. 4.

Fig. 5.

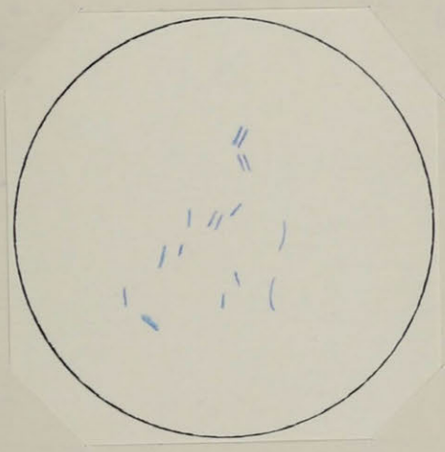


Fig. 6.

Fig. 7.

Fig. 8.

PLATE 17.

Mycobacterium coeliacum.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

Fig. 5. - 1 day. - Ziehl-Neelsen Stain.

Fig. 6. - 1 day. - Gabbet's Stain.

PLATE 17.

Fig. 1.

Fig. 2.

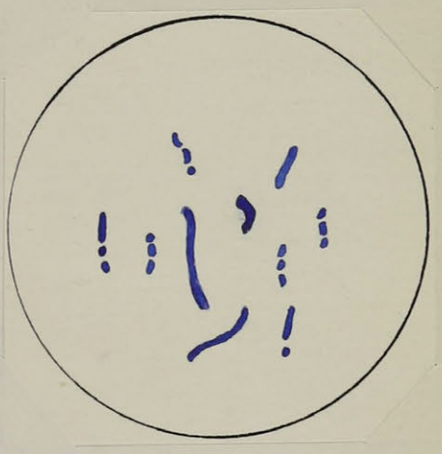


Fig. 3.

Fig. 4.

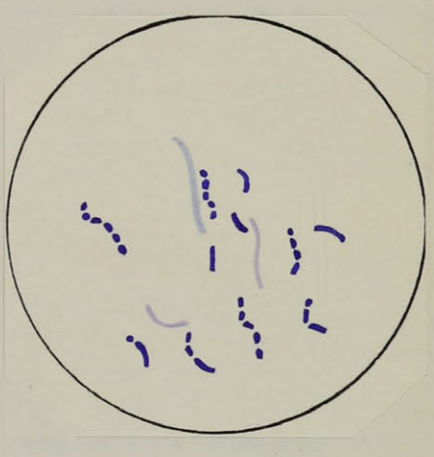


Fig. 5.

Fig. 6.

PLATE 18.

Mycobacterium convolutum.

(from B. P. Agar.)

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman

Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 14 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 14 days. Gabbet's Stain.

PLATE 18.

Fig. 1.

Fig. 2.

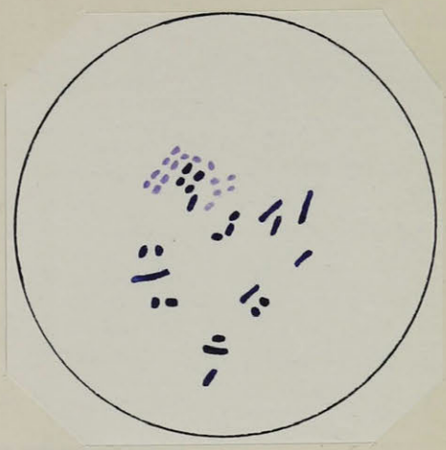


Fig. 3.

Fig. 4.

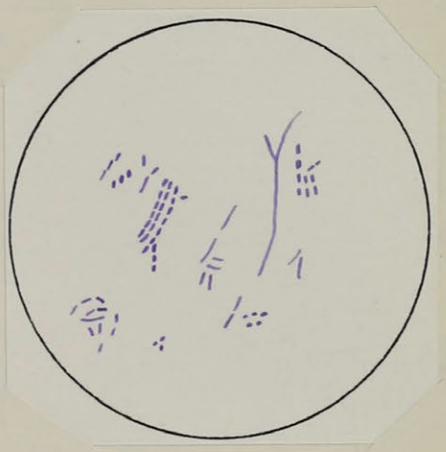


Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

PLATE 19.

Mycobacterium crystallophagum.

(From B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 30 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 30 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 30 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 30 days. Gabbet's Stain.

PLATE 19.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

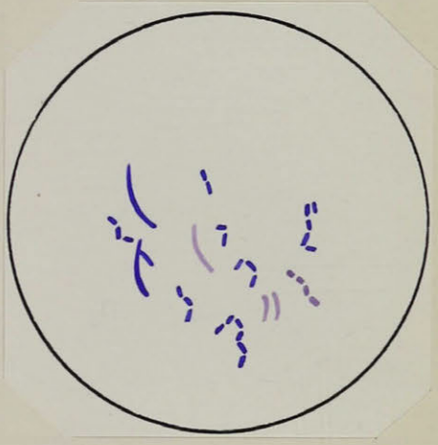


Fig. 5.

Fig. 6.

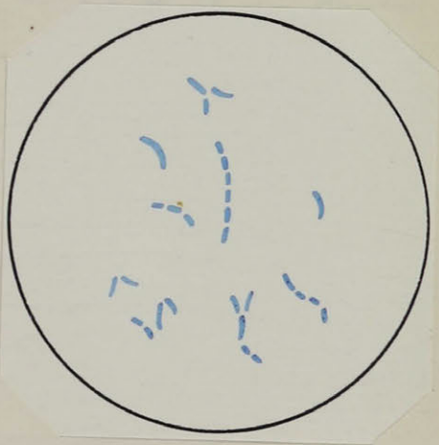


Fig. 7.

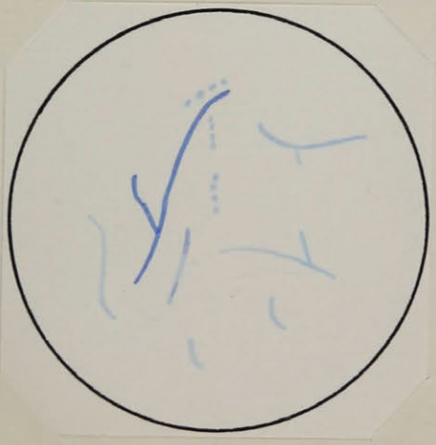


Fig. 8.

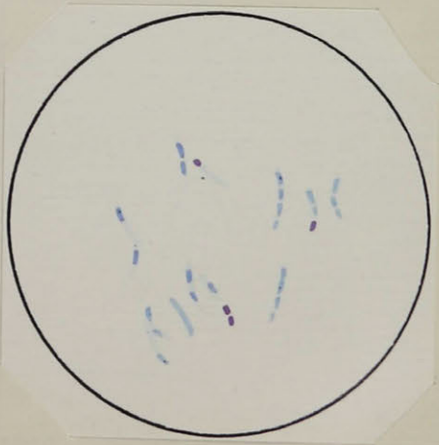


PLATE 20.

Mycobacterium erythropolis.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 14 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 14 days. Gabbet's Stain.

PLATE 20.

Fig. 1.

Fig. 2.

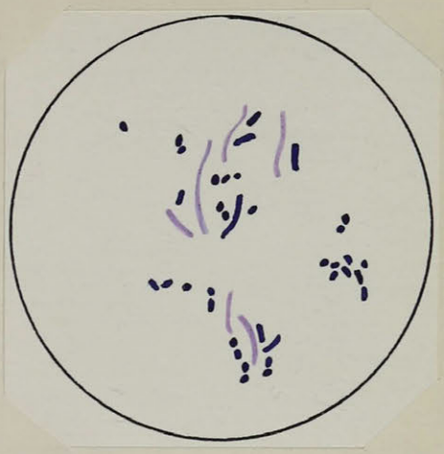


Fig. 3.

Fig. 4.

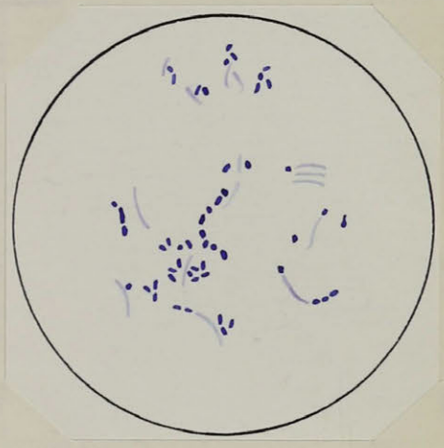


Fig. 5.

Fig. 6.

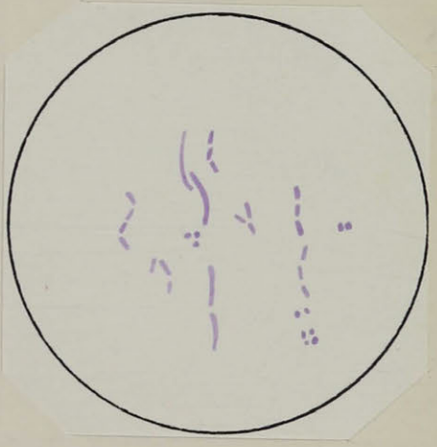


Fig. 7.

Fig. 8.

PLATE 21.

Mycobacterium globerulum.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 30 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 30 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 30 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 30 days. Gabbet's Stain.

PLATE 21.

Fig. 1.

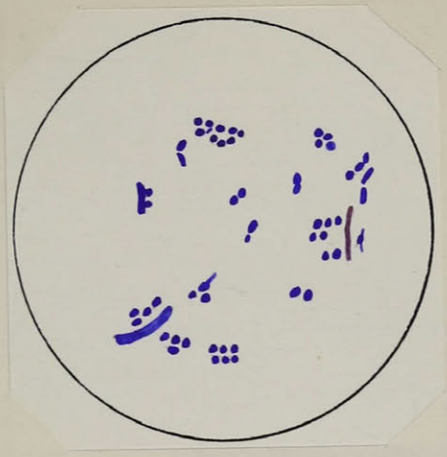


Fig. 2.

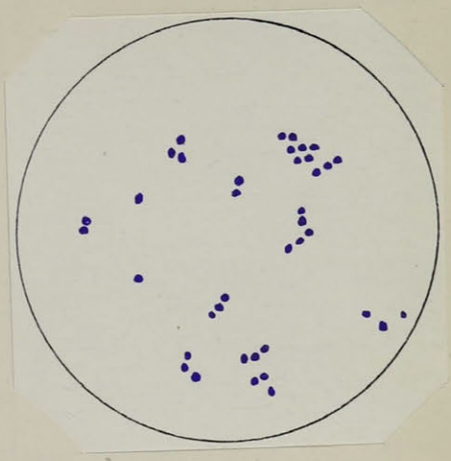


Fig. 3.

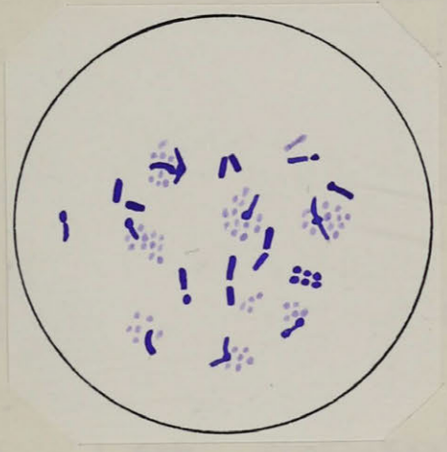


Fig. 4.

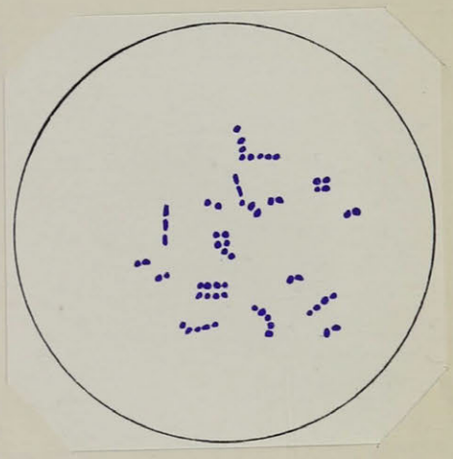


Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

PLATE 22.

Mycobacterium hyalinum.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. Kopeloff and Beerman Gram Stain.

Fig. 2. - 1 day. Hucker's Gram Stain.

Fig. 3. - 1 day. Ziehl-Neelsen Stain.

Fig. 4. - 1 day. Gabbet's Stain.

PLATE 22.

Fig. 1.

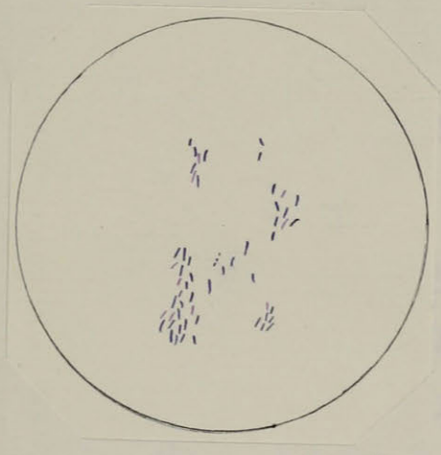


Fig. 2.

Fig. 3.

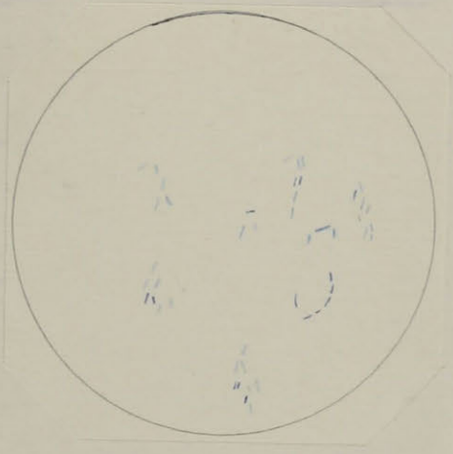


Fig. 4.

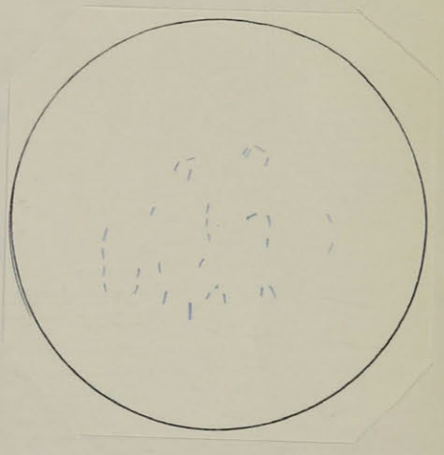


PLATE 23.

Mycobacterium lacticola.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 14 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 14 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 14 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 14 days. Gabbet's Stain.

PLATE 23.

Fig. 1.

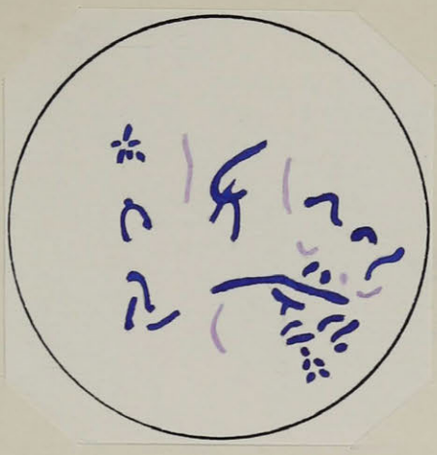


Fig. 2.

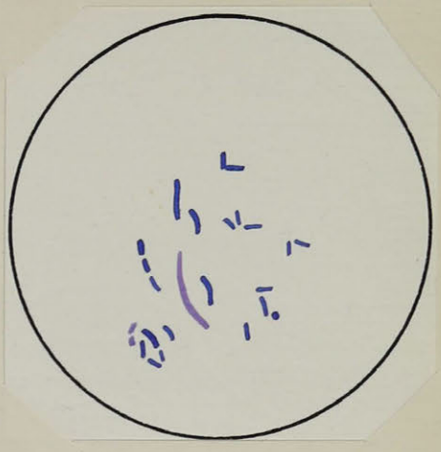


Fig. 3.

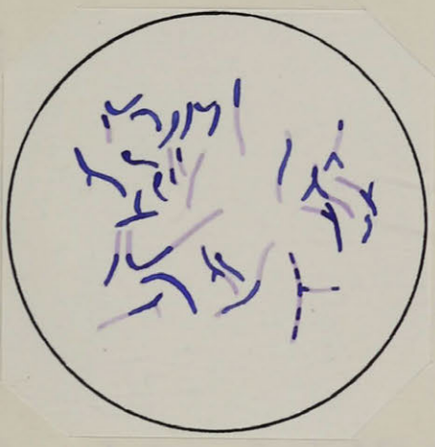


Fig. 4.

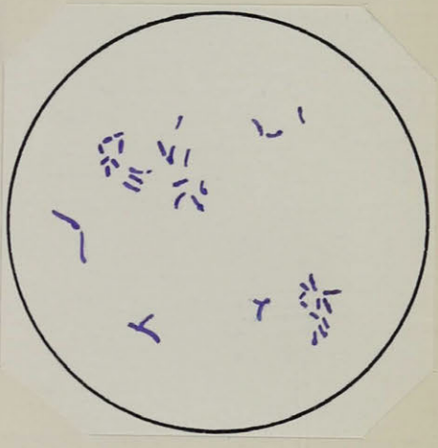


Fig. 5.

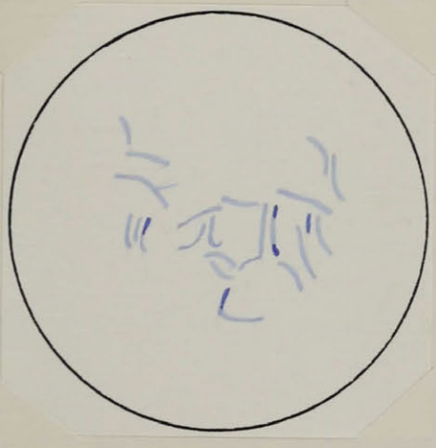


Fig. 6.

Fig. 7.

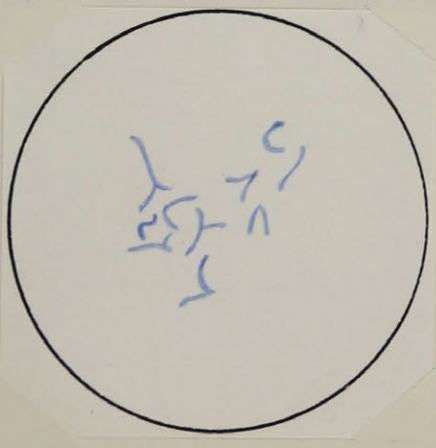


Fig. 8.

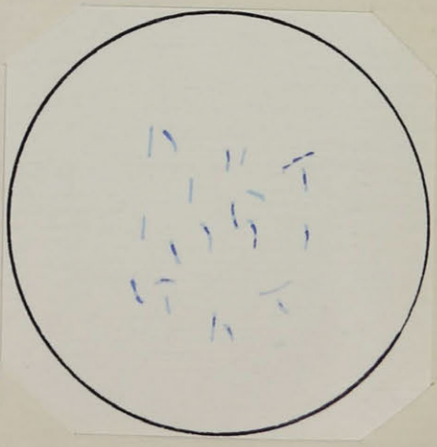


PLATE 24.

Mycobacterium luteum

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. - Kopeloff and Beerman Gram Stain.

Fig. 2. - 1 day. - Hucker's Gram Stain.

Fig. 3. - 1 day. Ziehl-Neelsen Stain.

Fig. 4. - 1 day. Gabbet's Stain.

Mycobacterium rubrum.

(from B. P. Agar - 1 day).

x 1200.

Fig. 5. - Kopeloff and Beerman Gram Stain.

FIG. 6. - Hucker's Gram Stain.

Fig. 7. - Ziehl-Neelsen Stain.

Fig. 8. - Gabbet's Stain.

PLATE 24.

Fig. 1.

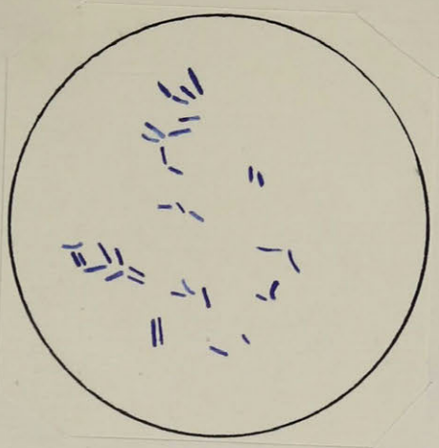


Fig. 2.

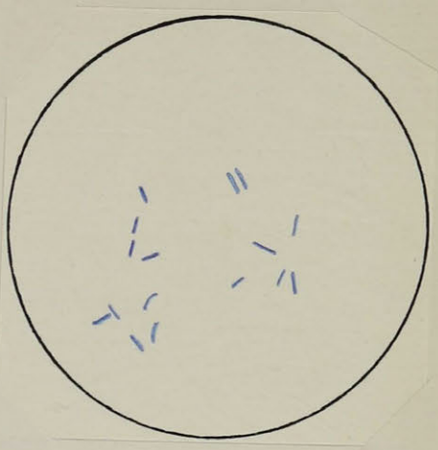


Fig. 3.

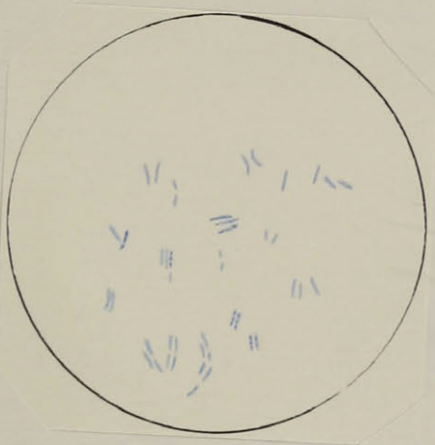


Fig. 4.

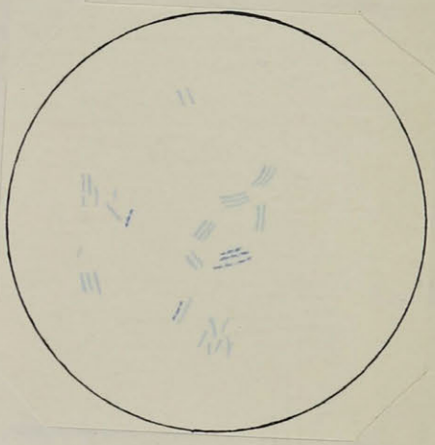


Fig. 5.

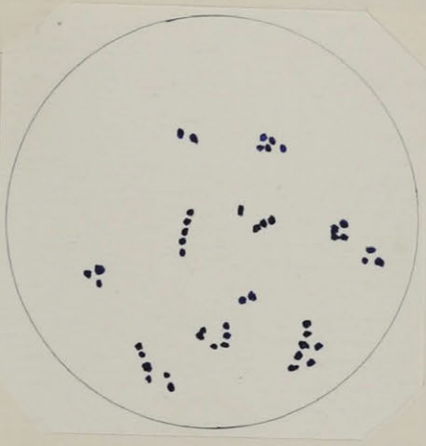


Fig. 6.

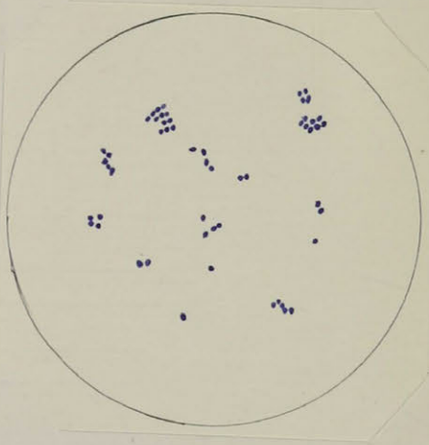


Fig. 7.

Fig. 8.

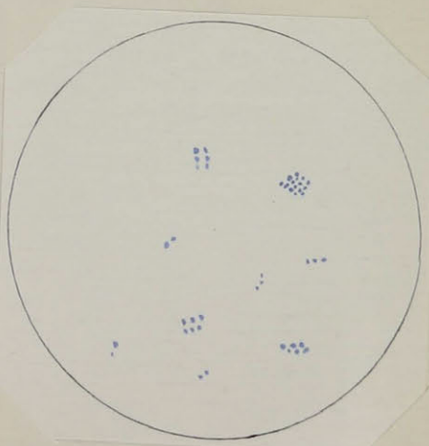


PLATE 25.

Mycobacterium opacum.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 30 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 30 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 30 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 30 days. Gabbet's Stain.

PLATE 25.

Fig. 1.

Fig. 2.

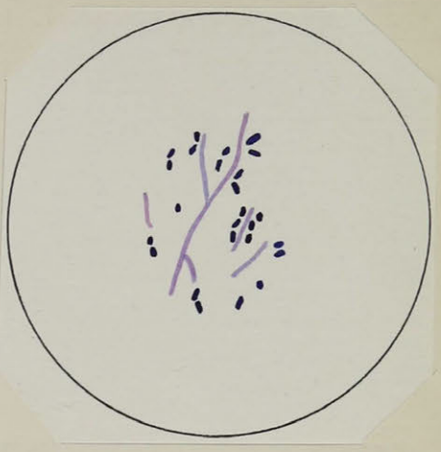


Fig. 3.

Fig. 4.

Fig. 5.

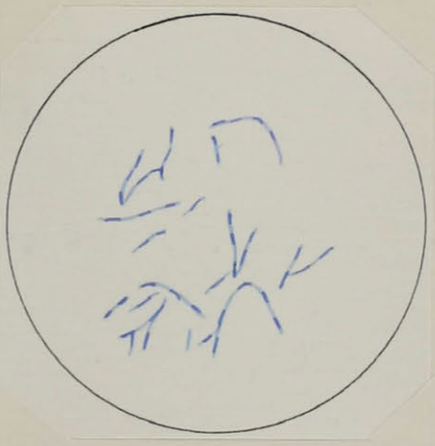


Fig. 6.

Fig. 7.

Fig. 8.

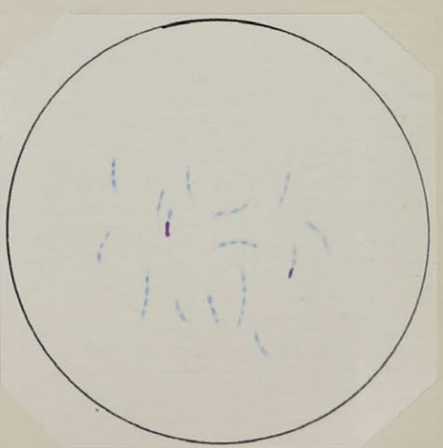


PLATE 26.

Mycobacterium salmonicolor.

(from B. P. Agar).

x 1200.

Fig. 1. - 1 day. & Fig. 2. - 30 days. Kopeloff and Beerman Gram Stain.

Fig. 3. - 1 day. & Fig. 4. - 30 days. Hucker's Gram Stain.

Fig. 5. - 1 day. & Fig. 6. - 30 days. Ziehl-Neelsen Stain.

Fig. 7. - 1 day. & Fig. 8. - 30 days. Gabbet's Stain.

PLATE 26.

Fig. 1.

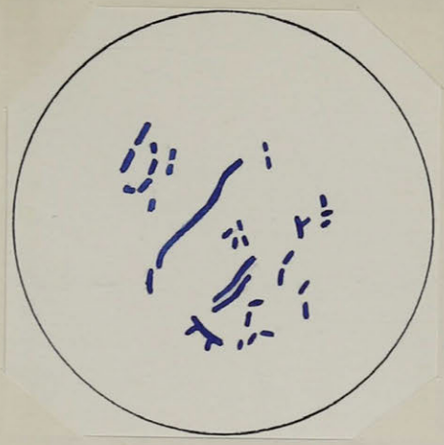


Fig. 2.

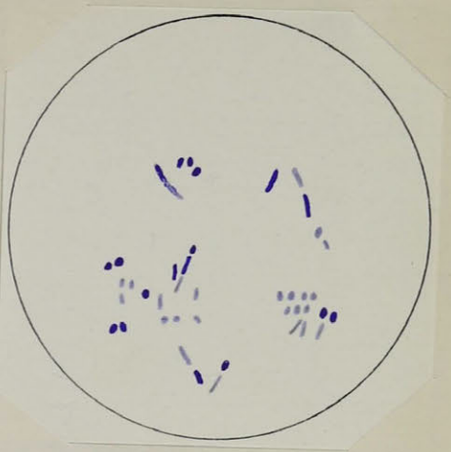


Fig. 3.

Fig. 4.

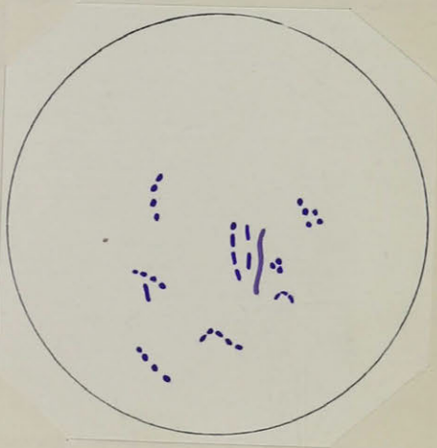


Fig. 5.

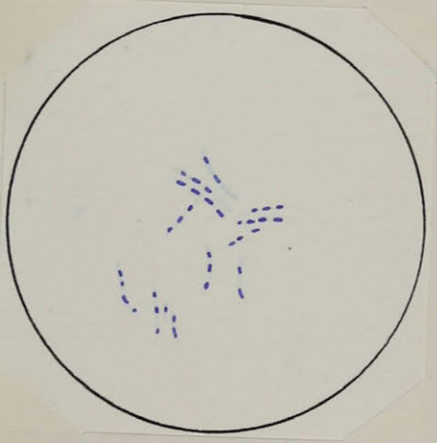


Fig. 6.

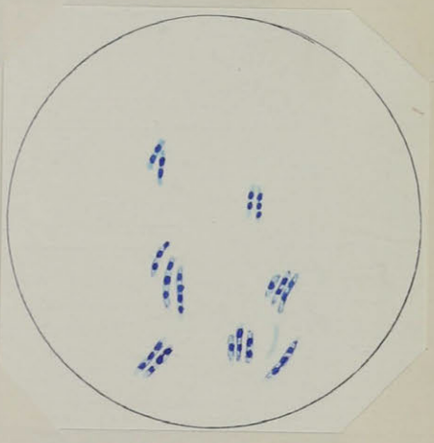


Fig. 7.

Fig. 8.

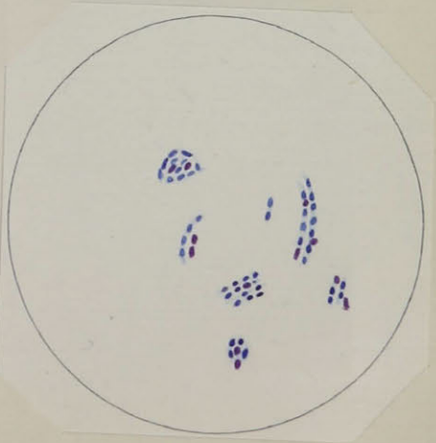


PLATE 27.

Fig. 1. On Dextrose Agar - 14 days.

A - M. tuberculosis hominis (No. 1 A.).

B - M. stercusis

C - M. actinomorphum

D - M. tuberculosis bovis.

Fig. 2. On Dextrose Agar - 14 days.

A - M. butyricum.

B - M. ranae

C - M. smegmatis.

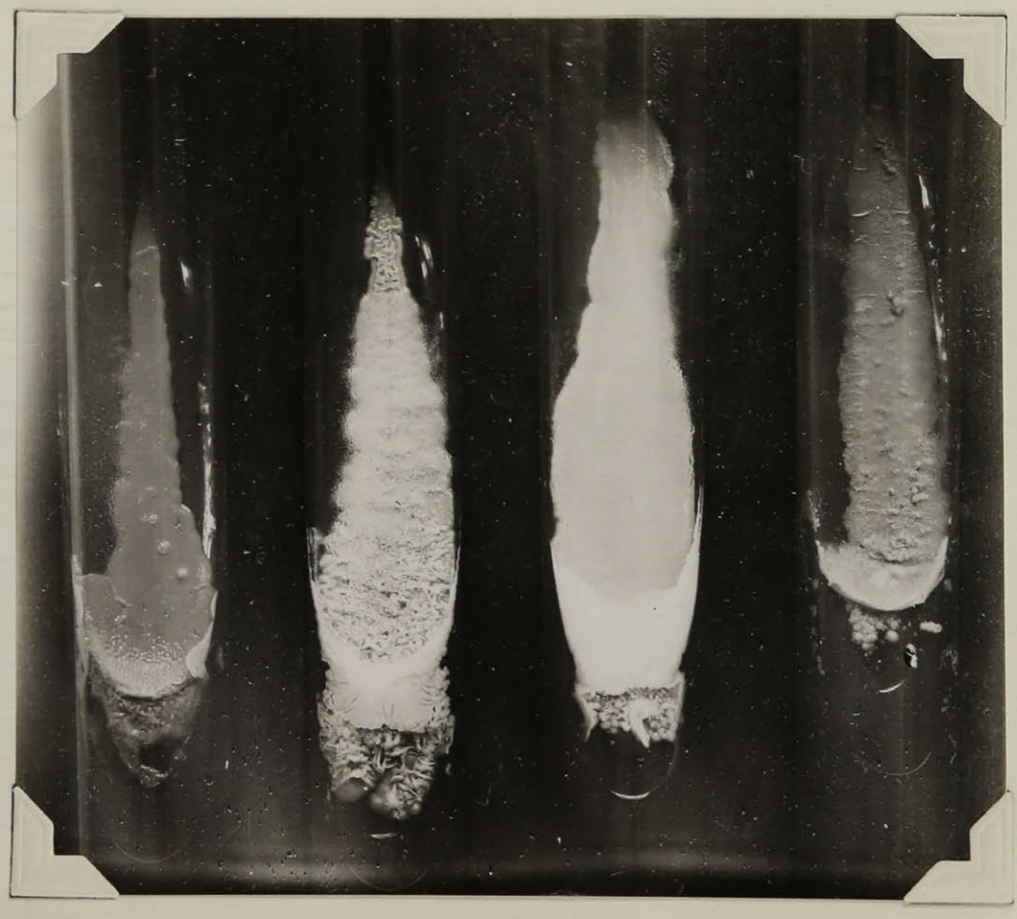
D - M. phlei.

PLATE 27.

Fig. 1.

A. B. C. D.

Fig. 2.



A. B. C. D.

PLATE 28.

Fig. 1. On Dextrose Agar - 14 days.

A - M. crystallophagum

B - M. graminis

C - M. coeliacum.

D - M. berolinensis.

Fig. 2. On Dextrose Agar - 7 days.

A - M. salmonicolor

B - M. rubrum

C - M. chelonei

D - M. graminis.

PLATE 28.

Fig. 1.

A. B. C. D.

Fig. 2.

A. B. C. D.

PLATE 29

Fig. 1 - On Dextrose Agar - 7 days.

A - M. thamnopheos.

B - M. convolutum

C - M. luteum

D - M. erythropolis

Fig. 2 - Glycerin Agar - 14 days.

A - M. avium.

B - M. convolutum

C - M. luteum

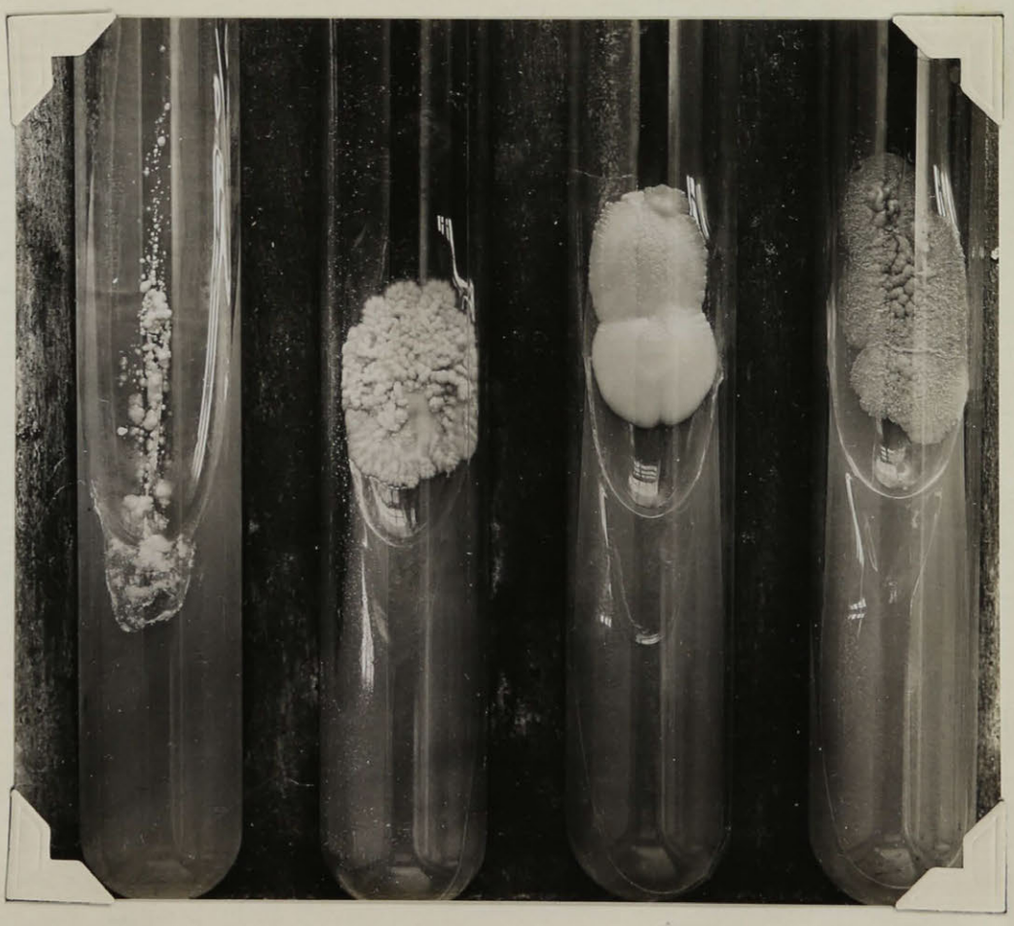
D - M. marinum.

PLATE 29.

Fig. 1.

A. B. C. D.

Fig. 2.



A. B. C. D.

PLATE 30.

Fig. 1 - On Dextrose Agar - 14 days.

A-- M. marinum

B - M. opacum

C - M. agreste

D - M. hyalinum

Fig. 2 - On Dextrose Agar - 7 days.

A - M. album

B - M. globerulum

C - M. piscium

D - M. lacticola.

PLATE 30.

Fig. 1.

A. B. C. D.

Fig. 2.

A. B. C. D.

PLATE 31.

Fig. 1 - 5% Glycerin Broth - 14 days.

A - M. tuberculosis bovis.

B - M. berolinensis.

C - M. smegmatis

D - M. ranae

Fig. 2 - 5% Glycerin Broth - 14 days.

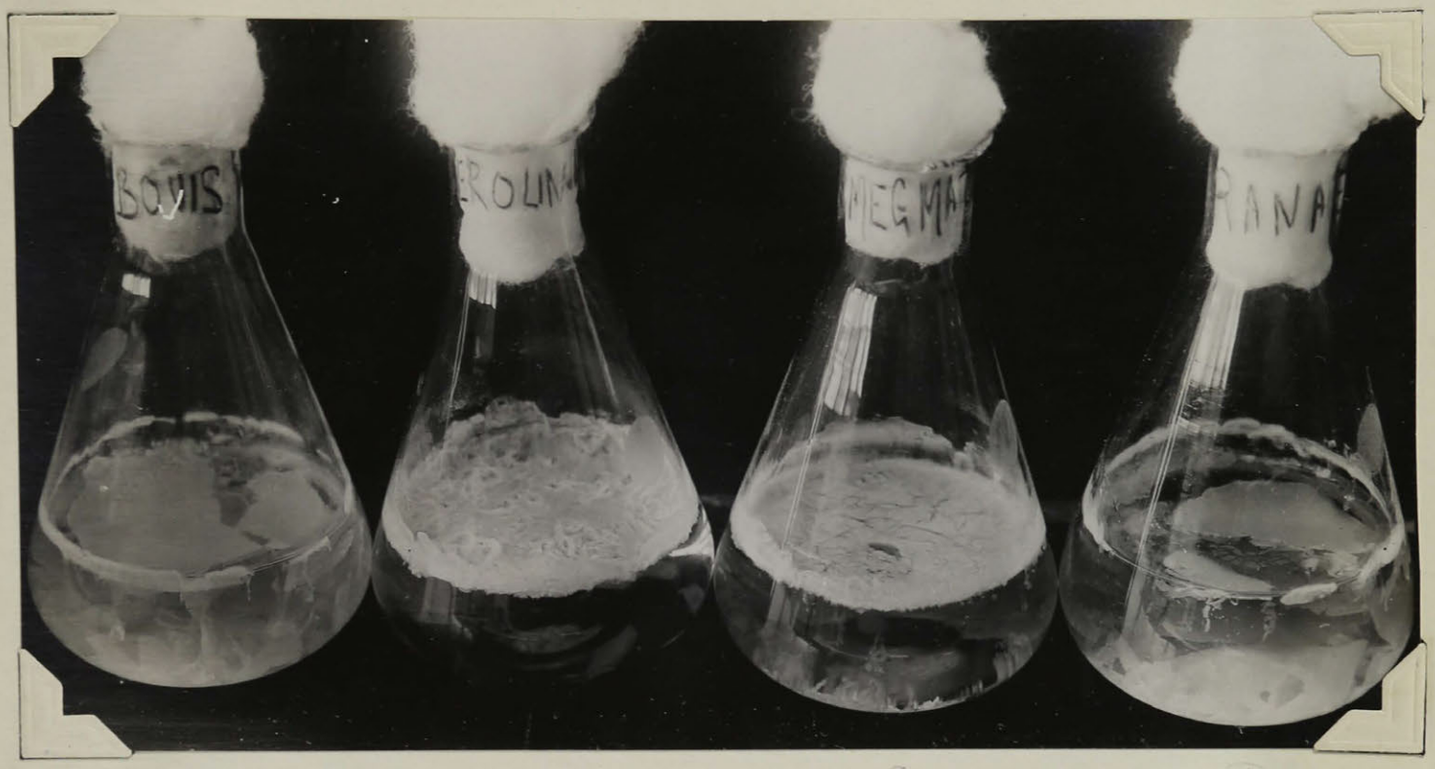
A - M. tuberculosis hominis (1 A.).

B - M. avium

C - M. rubrum

D - M. stercusis

PLATE 31.



A. B. C. D.

Fig. 1.

A. B. C. D.

Fig. 2.

PLATE 32.

Fig. 1. - 5% Glycerin Broth - 7 days.

A - M. opacum

B - M. graminis

C - M. hyalinum

D - M. thamnopheos.

Fig. 2. - 5% Glycerin Broth - 7 days.

A - M. butyricum

B - M. globerulum

C - M. marinum

D - M. chelonei

PLATE 32.

A. B. C. D.

Fig. 1.

A. B. C. D.

Fig. 2.

PLATE 33.

Fig. 1. - 5% Glycerin Broth - 14 days.

A - M. convolutum

B - M. phlei

C - M. luteum

Fig. 2. - 5% Glycerin Broth - 14 days.

A - M. actinomorphum

B - M. agreste

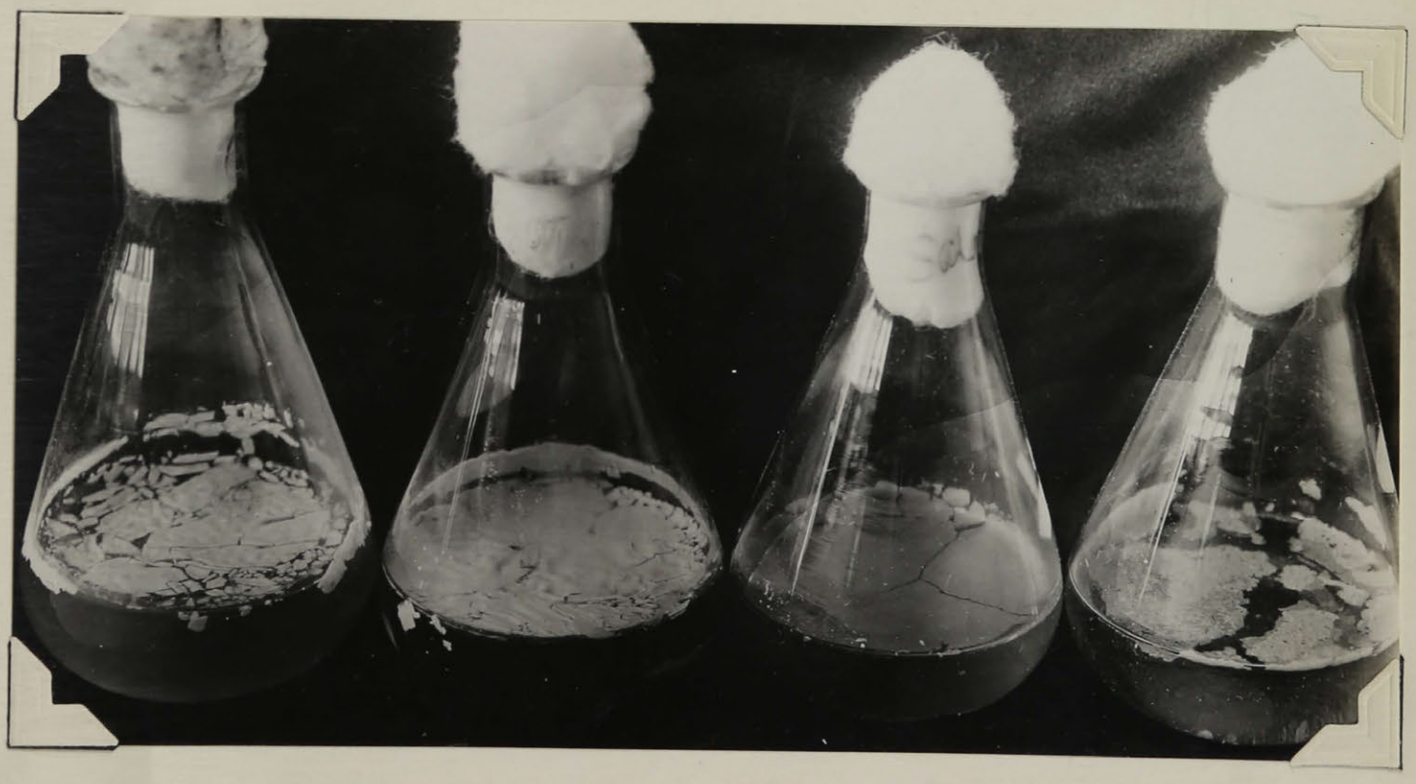
C - M. salmonicolor

D - M. crystallophagum

PLATE 33.

A. B. C.

Fig. 1.



A. B. C. D.

Fig. 2.