Published August 1, 1947

VIRULENCE AND MORPHOLOGICAL CHARACTERISTICS OF MAMMALIAN TUBERCLE BACILLI

By GARDNER MIDDLEBROOK, M.D., RENÉ J. DUBOS, PH.D., AND CYNTHIA PIERCE, PH.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

PLATES 19 AND 20

(Received for publication, May 9, 1947)

Although variations in the morphological characteristics of cultures of mammalian tubercle bacilli have long been known and extensively described, the relation of these characteristics to virulence remains uncertain. Many attempts have been made to correlate virulence and avirulence on the one hand with smoothness and roughness of colonial morphology on the other (1–5). Unfortunately, the unstable physical and chemical characteristics of the classical solid egg media have led to much confusion in terminology and description (6, 7).

The recent development of convenient techniques for the growth of tubercle bacilli in liquid and on solid media of relatively simple and reproducible composition (9–11) has permitted a reinvestigation of the morphology of these organisms. Moreover, the possibility of establishing in mice experimental tuberculous infections with small numbers of the bacilli (8) has allowed more accurate measurement and, therefore, a more thorough analysis of the relation of virulence to certain morphological characteristics of the culture.

Our study of the correlation between virulence and morphological characteristics has been based on the earlier investigations of Petroff, Steenken, and their associates (1, 12–16). By aging virulent cultures of mammalian tubercle bacilli on solid egg media, these investigators have obtained stable variant strains which are avirulent for guinea pigs. For example, the classical H37 strain has been dissociated into the highly virulent variant, H37Rv, and the stable avirulent variant, H37Ra, and certain consistently different cultural characteristics have been recognized between these two variants (17). This work has made available for comparative analysis of virulence and morphology two variant forms of the H37 strain as well as a number of other stable variants of virulent cultures of known virulence for the guinea pig.[1]

The present paper attempts to correlate certain differential morphological characteristics of these cultures with their virulence, measured either by the classical techniques in the guinea pig or by the recently described mouse infection tests (8).

[1] These cultures were obtained from the National Tuberculosis Association's Standard Culture Depot at Trudeau in the spring of 1946 and since that time have been transferred as routine in the Tween-albumin liquid medium.

Published August 1, 1947

Materials and Methods

The following strains of mammalian tubercle bacilli were used:

The two "extreme" variants of the classical H37 strain derived from a culture of a single cell: H37Rv, virulent, and H37Ra, avirulent (17).

H4Ra, reported to be avirulent for guinea pigs (14).

JH16Ra, reported to be avirulent for guinea pigs (14).

R1Rv, reported to possess low but definite virulence for normal guinea pigs and to be fatally virulent for silicotic guinea pigs, and its variant R1Ra, reported to be avirulent for guinea pigs (15).

A strain of BCG (BCG 317) obtained from the Henry Phipps Institute.

Ravenel, a classical virulent bovine strain.

Bovine 3817, a recently isolated virulent bovine strain.

Many strains recently isolated from sputum, spinal fluid, and blood of tuberculous patients.

All these strains were cultivated and transferred as routine in the previously described liquid medium containing 0.05 per cent Tween 80 and 0.2 to 0.5 per cent bovine serum albumin (fraction V) (8).

Virulence for mice was tested by intraperitoneal, intravenous, or intracerebral injection of 7 to 10 day old diffuse cultures in Tween-albumin liquid medium (8). Evaluation of virulence was based upon the ability of the culture to produce death, grossly visible lung lesions, and enlargement of the spleen, within 4 weeks after infection with various doses of living organisms.

Details concerning the preparation of basal liquid medium and its modifications have been described elsewhere (11 *a*).

Microscopic appearance of the cells growing submerged in liquid media was studied in the growths obtained by inoculating 0.1 cc. amounts of 6 to 10 day cultures into 5 cc. of basal medium containing various amounts of Tween 80 (0 to 0.08 per cent). Observations were also made on the morphological characteristics of the growth on the surface of liquid medium. This was prepared by adding to the basal medium 0.75 per cent glycerine (reagent or C.P. grades) before autoclaving and 0.75 per cent glucose after autoclaving (11 *a*). The medium was distributed in 200 cc. amounts in Blake bottles of 1 liter capacity. Each bottle was inoculated with 4 cc. of a 6 to 10 day old culture grown in basal medium containing 0.05 per cent Tween 80 and 0.3 per cent serum albumin. The Blake bottle was allowed to rest on its side for 2 days, during which time the bacteria multiplied against the glass on the bottom of the undisturbed vessel. This was then quickly tipped up and held in this position for a few seconds; much of the culture remained clinging to the side of the bottle. When the latter was returned to its horizontal position small islets of organisms were floated onto the surface of the medium and served to initiate a relatively rapid surface growth in the form of a pellicle.

For investigation of colonial morphology 0.1 cc. amounts of 10^{-3} dilutions of 6 to 10 day old cultures in the routine liquid medium were inoculated onto the surface of solid media containing 1.5 per cent agar and 0.5 per cent serum albumin. Glucose, oleic acid, and Tween 80 were added as described in the text.

Comparative Virulence of Mammalian Tubercle Bacilli for Mice and Guinea Pigs

It has been reported in the preceding paper that the two "extreme" variants of the H37 strain differ strikingly in virulence for mice (8). Table I summarizes similar observations concerning the comparative virulence of other representative strains of tubercle bacilli. While the H37Rv, Ravenel, and Bovine 3817

Published August 1, 1947

strains, which are virulent for guinea pigs, initiate a rapidly progressive infection in the relatively resistant Rockefeller Institute strain of mice, the three strains, H37Ra, JH16Ra, and H4Ra, are incapable of establishing a demonstrably progressive infection in the same strain of mice. Even more striking

TABLE I

Comparison of the Virulence for Mice of Cultures of Mammalian Tubercle Bacilli

Culture	Amount	Strain of mouse	Route of inoculation	No. of deaths in 4 wks.	Incidence and type of grossly visible lung lesions	Increase in size of spleen	Incidence of acid-fast rods on smear
	cc.						
H37Rv	0.5	R.I.	Intraperitoneal	0	Many, small, irregular, flat	++++	Lungs, many
	0.1	R.I.	Intravenous	All dead	Many discrete	++++	" "
	0.1×10^{-3}	R.I.	"	0	Moderate number, very small	++	" few
	0.1	dba	"	All dead in 1 wk.	Hemorragic lesions	±	" many
	0.1×10^{-7}	dba	"	0	Few large lesions	++	" "
	0.03×10^{-6}	dba	Intracerebral	0	Few lung lesions	+	Brain, many at 7 days Lungs, many at 4 wks.
H37Ra	1.0	R.I.	Intraperitoneal	0	None	0	Lungs, none
	0.1	R.I.	Intravenous	0	"	0	" rare
	0.5	C57	Intraperitoneal	0	"	0	" none
	0.1	dba	Intravenous	0	"	+++	" rare; spleen, none
	0.03	dba	Intracerebral	0	"	0	Brain, rare at 7 days
JH16Ra	0.2	R.I.	Intravenous	0	"	0	Lungs, rare
H4Ra	0.2	R.I.	"	0	"	0	" "
R1Rv	0.1	R.I.	"	0	Scattered, small	++	" moderate
BCG 317	0.1	R.I.	"	0	" "	++	" "
Ravenel	0.1×10^{-3}	R.I.	"	0	Moderate number, very small	++	" few
Bovine 3817	0.1×10^{-3}	R.I.	"	0	Moderate number, small	++	" moderate

R.I. = Rockefeller Institute strain (albino)—resistant to tuberculous infection.
C57 and dba (pigmented)—susceptible to tuberculous infection.
5 or 6 mice were used in each group tested.

differences between the virulent and avirulent variants are revealed by intracerebral inoculation into the susceptible dba strain of mice. Virulent organisms in very small doses (for example, 0.03×10^{-6} cc. of a culture of the virulent variant of H37) are capable of early and rapid multiplication intracerebrally and cause lesions in the lungs within 4 weeks. The avirulent variant, on the contrary, in a dose 100,000 times this amount, does not appear to multiply in the brain and produces no secondary lesions. Except when the infection is overwhelmingly acute and fatal, or when it is initiated by very small doses of

bacilli, virulent variants produce marked enlargement of the spleen; this is never observed in the Rockefeller Institute strain of mice inoculated with avirulent variants.

As indicated in Table I, the R1Rv and BCG strains have been observed consistently to produce within 4 weeks small, but macroscopically visible, pulmonary lesions and some enlargement of the spleen in the relatively resistant Rockefeller Institute strain of mice infected intravenously with 0.1 cc. of undiluted culture. These two strains of mammalian tubercle bacilli possess, therefore, low but definite virulence, since they are able to multiply *in vivo*, at least for a period of time, and to produce lesions in mice. Similarly, the R1Rv strain is known to possess a low degree of virulence for the normal guinea pig (15, 16); the virulence for the guinea pig of the BCG strain used in the present studies has not been determined.

On the basis of the observations which have just been reported it appears justifiable to conclude that the virulence of mammalian tubercle bacilli, as measured by mouse infection tests, correlates well with the degrees of virulence determined by the classical techniques in the guinea pig.

Comparative Morphological Characteristics of Strains of Tubercle Bacilli Endowed with Different Degrees of Virulence

1. Comparison of H37Rv and H37Ra.—For convenience of presentation, observations on the two "extreme" variants, virulent and avirulent, of the classical H37 strain will be reported first. Studies on other strains will be described by reference to these two variants.

Inoculation of the basal medium containing serum albumin and no more than 0.02 per cent Tween 80 with a culture (in Tween-albumin medium) recently isolated from an infected mouse gives rise within a few days to a growth consisting of bundles, ropes, or cords of strongly acid-fast bacilli in which the orientation of the long axis of each cell is parallel to the long axis of the cord. Figure 1 *b* illustrates this type of cellular arrangement. In the absence of Tween 80 or in the presence of less than 0.01 per cent of this substance, the culture appears as a maze of intertwined serpentine cords when viewed under low power magnification.

The tendency of the virulent variant to form cords can be completely inhibited by cultivation in media containing 0.05 per cent or more of Tween 80; under these conditions the culture becomes highly diffuse and consists predominantly of isolated bacterial cells. Transfer of this highly dispersed culture back to medium containing no Tween 80 or a low concentration of this substance results again in growth in the form of cords.

That cord formation is not an artifact of cultivation *in vitro* is indicated by the finding of short but definite cords and bundles of tubercle bacilli arranged in parallel in the brain tissue of mice infected intracerebrally with high dilutions

Published August 1, 1947

of a diffuse culture of H37Rv. Cords are also readily demonstrable in the yolk sacs of chick embryos infected with H37Rv or with other virulent cultures of mammalian tubercle bacilli.

In contrast to these morphological characteristics of the virulent variant of H37, the completely avirulent variant, H37Ra, has never been observed to form definite cords under any condition of growth. It grows, as illustrated in Fig. 1 *a*, in non-oriented clumps the size of which depends upon the age of the culture and the concentration of Tween 80 in the medium. Furthermore, the cells of H37Ra are less acid-fast, taking the methylene blue counterstain more readily than the cells of the virulent culture.

On the surface of Tween–albumin-agar media H37Rv, recently isolated from infected animals, gives rise to the type of colonies illustrated in Fig. 2 *b*. The colonies are flat and highly translucent, and display a serpentine structure in the presence of low concentrations of Tween 80. On the other hand the use of higher concentrations of Tween 80 decreases the tendency to serpentine growth on the surface of agar media as it inhibits the formation of cords in liquid media.

When inoculated onto the surface of agar media containing more than 0.005 per cent Tween 80 the avirulent variant grows in the form of the smooth, raised, opaque colonies illustrated in Fig. 2 *a*.

The most striking differences between the two variants of H37 are brought out by studying the colonial morphology on the surface of agar medium containing 0.5 per cent serum albumin and no Tween. As shown in Figs. 3 *a* and 3 *b*, these fundamental differences are independent of the size of the colony. The ability of the virulent variant to form serpentine cords which tend to spread out over the surface of the agar appears to account for all the gross morphological differences between the colonies of the two variants.

The differential morphological characteristics of the growth of the two variants of H37 on the surface of liquid medium, previously observed by Steenken (17, 18), have also been recognized in our studies. The virulent variant has a marked tendency to form a thin veil which spreads uniformly and rapidly over the entire surface of the liquid medium and climbs high on the sides of the glass container; the avirulent variant, in contrast, has much less tendency to spread and heaps up in more or less discrete islands which do not coalesce for long periods. Microscopic examination of fixed specimens of surface pellicles reveals the presence of the serpentine cords in the virulent culture and the absence of any consistent orientation in the growth of the avirulent variant. Thus the difference in macroscopic morphology on the surface of liquid medium is a reflection of the same basic difference observable microscopically in submerged culture and on solid agar media.

The morphological characteristics described above were constant over the pH range 6.0 to 7.0, and were not affected by the addition of up to 0.5 per cent glucose to the medium. Oleic acid added in a final concentration of 0.005 per

Published August 1, 1947

cent to albumin-agar (11 *a*) stimulates the growth of both variants of the H37 strain but has no effect on the fundamental differences in their morphological properties.

2. Characteristics of Other Virulent Strains of Mammalian Tubercle Bacilli.—All strains of tubercle bacilli freshly isolated from human pathological materials which have thus far been examined in our laboratory possess high virulence for the mouse and exhibit the morphological characteristics of the virulent H37Rv culture. Fig. 4 illustrates the typical morphology of a primary culture in Tween-albumin liquid medium of tubercle bacilli recovered from the sputum of a patient with pulmonary tuberculosis. The morphological characteristics of the virulent bovine strains, Ravenel and Bovine 3817, are also identical with those of the virulent variant of the H37 strain with respect to cord formation and strong acid fastness.

3. Characteristics of Other Completely Avirulent Variants.—The two variants, JH16Ra and H4Ra, which possess the same very low order of virulence as the H37Ra strain, exhibit also identical morphological characteristics with respect to their failure to form cords and their low degree of acid fastness in Tween-albumin medium. Their colonies on the surface of solid agar media are similar to those of H37Ra (see Fig. 5).

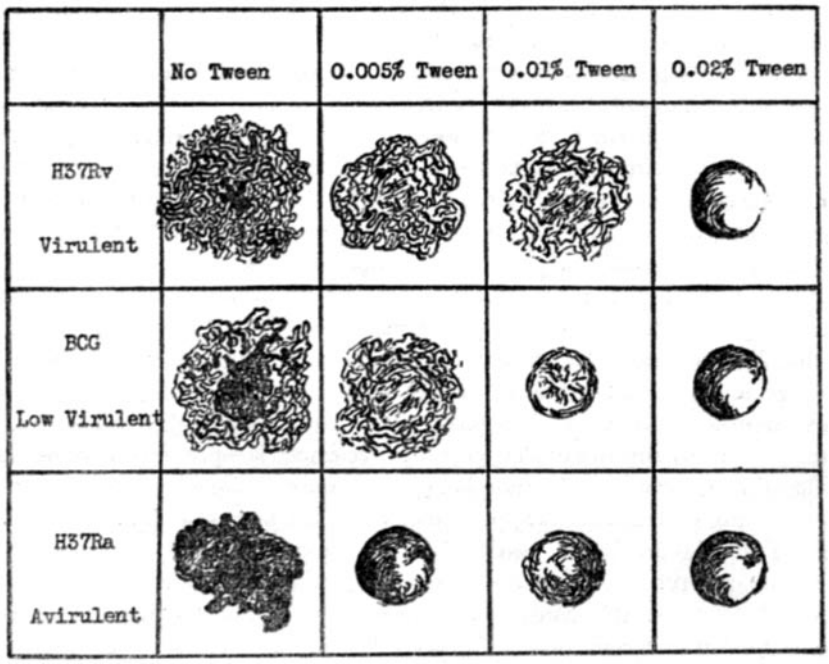
All avirulent variants thus far studied grow somewhat more slowly than do the virulent strains in the depth of Tween-albumin liquid media and on the surface of agar media. This is correlated with their slower growth on egg media (19). Moreover, it is more difficult to initiate growth of these avirulent variants in Tween-albumin medium than is the case with virulent strains, a fact particularly striking in the case of R1Ra, the completely avirulent variant of the R1Rv strain. These differences may be due to greater susceptibility of avirulent variants to the toxic effect of the unesterified oleic acid which contaminates commercial preparations of Tween 80 (20). It is worth noting, however, that once growth of an avirulent variant has been initiated in the Tween-albumin liquid medium, it is not difficult to maintain. Furthermore, the growth requirements of completely avirulent variants have not been observed by us or by previous investigators to be more exacting than those of highly virulent parent strains.

5. Morphological Characteristics of Two Strains of Low Virulence.—The R1Rv strain and the BCG 317 strain have been found to possess low but definite virulence for the mouse. Observations on the morphology of these strains reveal that they are more acid-fast than the completely avirulent variants but less acid-fast than highly virulent cultures grown in the Tween-albumin medium. They form cords in liquid medium in the absence of Tween 80 but not in media containing more than 0.01 per cent of this substance. Similarly, they give rise to flat, spreading colonies with serpentine structure on the surface of the albumin-agar medium containing no Tween 80; however, with 0.01 per cent, a concentration which has little if any effect on the colonial morphology of the fully virulent variants, the colonies of the R1Rv and BCG cultures are raised

Published August 1, 1947

and opaque like those of the completely avirulent variants in the presence of Tween 80. This effect is illustrated in Figs. 6 *a* and 6 *b* and Figs. 7 *a* and 7 *b*. Finally, BCG and R1Rv form on the surface of the liquid medium thin veils which consist of serpentine interconnected cords similar to those of the fully virulent strains.

Thus R1Rv and the BCG culture which we have studied behave as intermediates between the avirulent and fully virulent cultures of tubercle bacilli,



TEXT-FIG. 1. Representation of effect of Tween 80 on colonial morphology of typica mammalian strains.

with respect both to their virulence for the mouse and to their morphological properties.

The accompanying schema (Text-fig. 1) illustrates the observations concerning the colonial morphology of strains of widely differing virulence on the surface of the albumin-agar medium and the differential effect of Tween 80 on colonial structure.

DISCUSSION

The observations presented here demonstrate that the relative degrees of virulence of cultures of mammalian tubercle bacilli for mice correspond closely to the virulence of the same strains for guinea pigs. This correlation justifies

Published August 1, 1947

the use of the mouse for the study of the relative virulence of cultures of these microorganisms.

It has been recorded by many observers that cultures of mammalian tubercle bacilli which are virulent for the guinea pig give a spreading, veil-like, growth on the surface of liquid media whereas avirulent strains are characterized by a raised, non-spreading type of growth (1–3, 25–28). Moreover, these same observers have shown that when cultures of BCG, which gave the latter type of growth, became dissociated by any technique resulting in cultures possessing virulence of demonstrable degree, the virulent variants exhibited spreading growth on the surface of liquid media.

That cultures of mammalian tubercle bacilli can grow in the form of microscopic serpentine cords has also been frequently observed (21–24). Our observations establish a correlation between this characteristic microscopic morphology and the virulence of the strain. It has been shown, furthermore, that the tendency of cultures of virulent tubercle bacilli to spread on the surface of liquid media and on solid media is also directly correlated with their microscopically demonstrable property of formation of cords.

It is true that certain rapidly growing non-pathogenic mycobacterial cultures can also grow as thin veils and form serpentine cords on the surface of liquid media (29, 30). However, these cultures differ in so many other respects from pathogenic strains and avirulent variants of the latter that they need not be considered here. Although it is possible that there may be discovered, in the future, avirulent cultures of slow growing mycobacteria—presumably of pathogenic origin—exhibiting the morphological characteristics of virulent strains, the following generalizations appear justified on the basis of published descriptions of cultures and of our own studies: On the one hand, all virulent strains of mammalian tubercle bacilli are strongly acid-fast and always produce microscopically demonstrable cords when grown under certain specific, readily reproducible cultural conditions. On the other hand, cultures of slow growing mycobacteria which fail to form cords under the same cultural conditions and which, therefore, grow in the form of heaped, non-spreading, pellicles on the surface of liquid media, possess in our experience and in the recorded experience of other investigators very low virulence or no demonstrable virulence. *Thus the ability of cultures of mammalian tubercle bacilli to form cords under the specific conditions of cultivation described here appears to be an essential accompaniment of virulence.* It is tempting to postulate that in analogy with available knowledge concerning other bacterial species, the gross and microscopic morphological differences between the cultures of virulent and avirulent variants of mammalian tubercle bacilli reflect specific immunochemical differences which, when identified, will assist in the understanding of the pathogenesis and immunology of tuberculous infections.

SUMMARY AND CONCLUSIONS

Experimental infection of the mouse can be used for the determination of virulence of cultures of mammalian tubercle bacilli. The relative virulence of such cultures for the mouse is approximately the same as for the guinea pig.

Cultures of virulent and avirulent variants of mammalian tubercle bacilli grown in the depth of Tween 80-albumin liquid medium, on the surface of solid agar modifications of this medium, and on the surface of a liquid modification of this medium exhibit consistent morphological differences. All virulent cultures tend to form microscopically demonstrable serpentine cords of varying thickness and length consisting of highly acid-fast bacilli oriented in parallel along the long axis of the cord. The formation of cords appears to be an important factor in conditioning the ability of cultures to spread on the surface of liquid and solid media. It can be inhibited by the addition to the medium of the surface-active water-dispersible oleic acid ester, Tween 80. Avirulent variant bacilli grow in a more or less non-oriented fashion. They have never been observed to form cords under any condition of growth and are much less acid-fast than the virulent cultures when grown in Tween-albumin medium.

Two strains of mammalian tubercle bacilli which are intermediate in degree of virulence between the fully virulent and the avirulent variants also exhibit intermediate morphological characteristics.

BIBLIOGRAPHY

1. Petroff, S. A., Branch, A., and Steenken, W., *Am. Rev. Tuberc.*, 1929, **19**, 9.
2. Reed, G. B., and Rice, C. E., *Canad. J. Research*, 1931, **5**, 111.
3. Smithburn, K. C., *J. Exp. Med.*, 1935, **62**, 645.
4. Frimodt-Möller, J., Dissociation of Tubercle Bacilli, London, H. K. Lewis & Co., Ltd., 1939.
5. Alexander-Jackson, E., *Am. Rev. Tuberc.*, 1936, **33**, 767.
6. Steenken, W., Jr., *Am. Rev. Tuberc.*, 1940, **42**, 422.
7. Smithburn, K. C., *Am. Rev. Tuberc.*, 1937, **36**, 637.
8. Pierce, C. H., Dubos, R. J., and Middlebrook, G., *J. Exp. Med.*, 1947, **86**, 159.
9. Dubos, R. J., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58**, 361.
10. Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.
11. Dubos, R. J., Davis, B. D., Middlebrook, G., and Pierce, C. H., *Am. Rev. Tuberc.*, 1946, **54**, 204.

11 *a*. Dubos, R. J., and Middlebrook, G., *Am. Rev. Tuberc.*, in press.

12. Steenken, W., Jr., *Am. Rev. Tuberc.*, 1938, **38**, 777.
13. Steenken, W., Jr., and Smith, M. M., *Am. Rev. Tuberc.*, 1938, **38**, 514.
14. Steenken, W., Jr., and Gardner, L. U., *Yale J. Biol. and Med.*, 1943, **15**, 393.
15. Steenken, W., Jr., and Gardner, L. U., *Am. Rev. Tuberc.*, 1946, **54**, 51.
16. Dowd, G. R., *Am. Rev. Tuberc.*, 1935, **32**, 50.
17. Steenken, W., Jr., and Gardner, L. U., *Am. Rev. Tuberc.*, 1946, **54**, 62.
18. Steenken, W., Jr., *J. Biol. Chem.*, 1941, **141**, 91.

19. Steenken, W., Jr., personal communication.
20. Davis, B. D., and Dubos, R. J., *Arch. Biochem.*, 1946, **11**, 201.
21. Maximow, A., *Ann. Inst. Pasteur*, 1928, **42**, 225.
22. Nedelkovitch, J., *Ann. Inst. Pasteur*, 1936, **57**, 171.
23. Pryce, D. M., *J. Path. and Bact.*, 1941, **53**, 327.
24. Yegian, D., and Porter, K. R., *J. Bact.*, 1944, **48**, 83.
25. Petroff, S. A., and Steenken, W., Jr., *J. Exp. Med.*, 1930, **51**, 831.
26. Oatway, W., and Steenken, W., Jr., *Am. Rev. Tuberc.*, 1937, **35**, 354.
27. Sasano, K. T., and Medlar, E. M., *Tubercle*, 1931, **12**, 214.
28. Reed, G. B., Rice, C. E., and Orr, J. H., *Tr. Nat. Tuberc. Assn.*, 1932, 28th Annual Meeting, 147.
29. Bretey, J., Browaeys, J., and Dervichian, D., *Ann. Inst. Pasteur*, 1945, **71**, 233.
30. Yegian, D., personal communication.

EXPLANATION OF PLATES

The photographs were made by Mr. Joseph B. Haulenbeek.

PLATE 19

FIG. 1 *a*. H37Ra. Ziehl-Neelsen stained smear of a 7 day old culture in liquid medium containing 0.02 per cent Tween 80 and 0.5 per cent serum albumin. Note the lack of orientation in the arrangement of the cells of this avirulent strain. × 1000.

FIG. 1 *b*. H37Rv. Ziehl-Neelsen stained smear of a 7 day old culture in liquid medium containing 0.02 per cent Tween 80 and 0.5 per cent serum albumin. This culture was recently isolated from an experimentally infected mouse. Note the tendency to the formation of cords. × 1000.

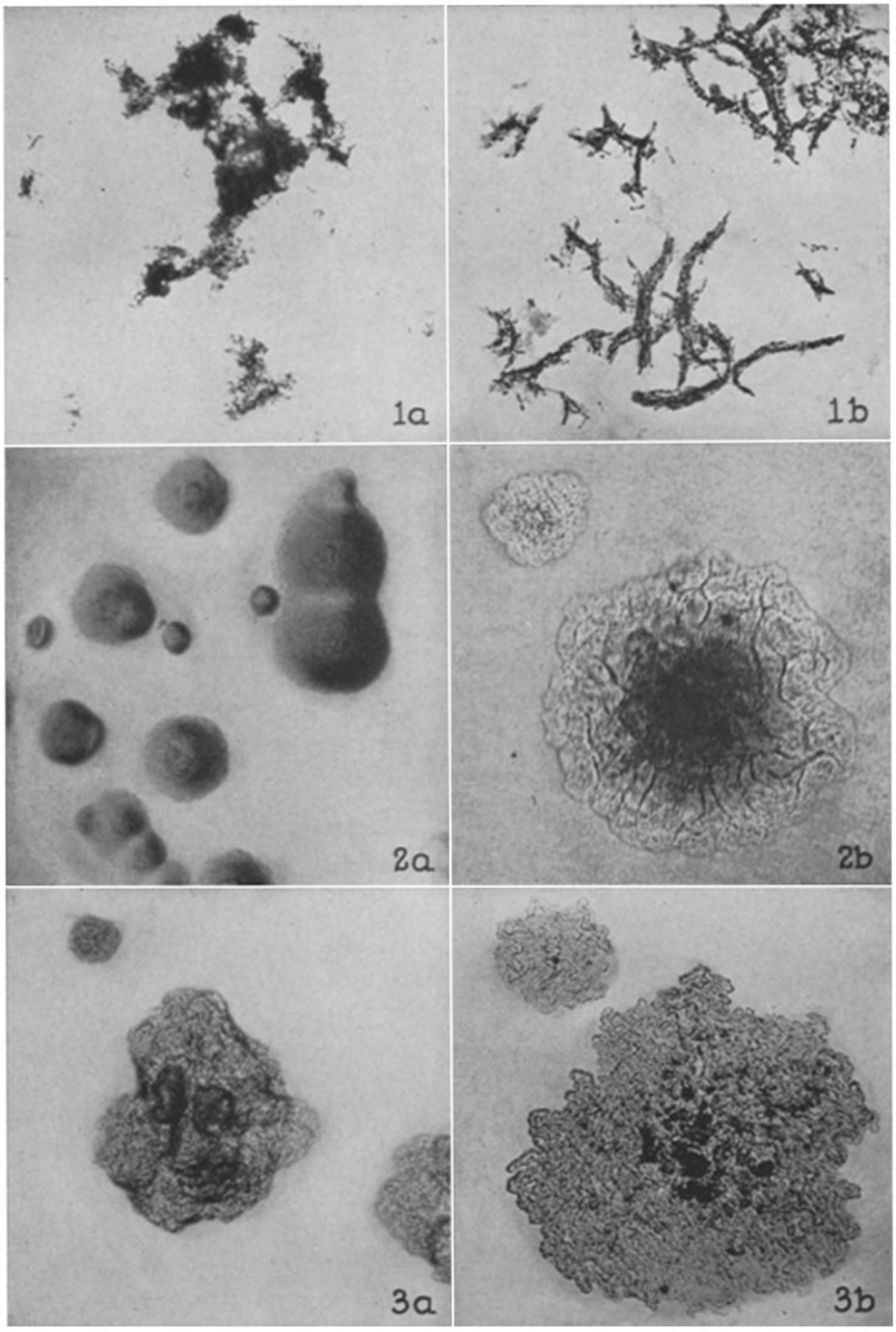
FIG. 2 *a*. H37Ra. 12 day old culture on the surface of the agar medium containing 0.01 per cent Tween 80 and 0.5 per cent serum albumin. The colonies are smooth surfaced, raised, and opaque. × 90.

FIG. 2 *b*. H37Rv. 12 day old culture on the surface of the agar medium containing 0.01 per cent Tween 80 and 0.5 per cent serum albumin. The colonies are flat and translucent, and have serpentine markings. × 90.

FIG. 3 *a*. H37Ra. 12 day old culture on the surface of the agar medium containing 0.5 per cent serum albumin and no Tween. Note the non-oriented structure of the colonies; the colonies are heaped-up and have little tendency to spread out over the surface of the medium. × 90.

FIG. 3 *b*. H37Rv. 12 day old culture on the surface of the agar medium containing 0.5 per cent serum albumin and no Tween. The colonies have a serpentine structure; cords are visible in the form of loops at the thin undulate margins; and they are flat because of their tendency to spread out over the surface of the medium. × 90.

Published August 1, 1947



(Middlebrook *et. al.*: Virulence and morphology of mycobacteria)

Published August 1, 1947

PLATE 20

FIG. 4. Ziehl-Neelsen stained smear of a primary culture of sputum in Tween-albumin liquid medium. The tubercle bacilli have grown in the form of intertwined serpentine cords in the depth of the liquid medium. × 155.

FIG. 5. H4Ra. Colonies of an avirulent culture of tubercle bacilli on the agar medium containing 0.5 per cent albumin and only 0.005 per cent Tween. Even in the presence of a very small amount of Tween, smooth and almost hemispherical colonies are characteristic of avirulent variants. × 125.

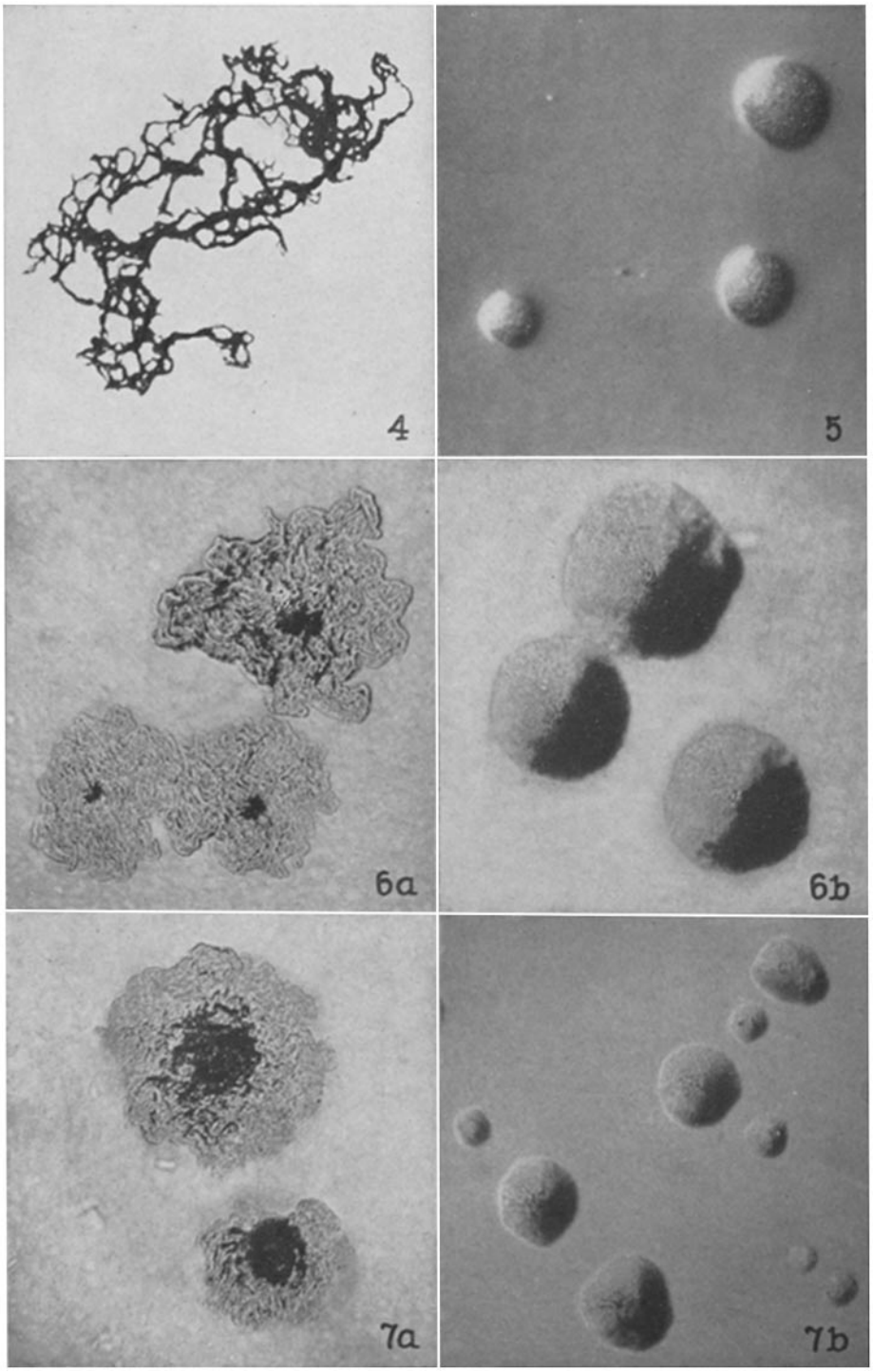
FIG. 6 *a*. R1Rv. Flat and spreading colonies of a low virulent strain on the surface of the agar medium containing 0.5 per cent albumin and no Tween. The cord structure of the colonies is visible. × 125.

FIG. 6 *b*. R1Rv. Colonies on the surface of the agar medium containing 0.5 per cent albumin and 0.01 per cent Tween 80; for comparison with Fig. 6 *a*; the small amount of Tween has inhibited the formation of cords and the colonies are raised like those of avirulent strains growing in the presence of the same amount of Tween. × 125.

FIG. 7 *a*. BCG 317. Colonies on the surface of the agar medium containing 0.5 per cent albumin and no Tween. The serpentine structure of the colonies is evident. Compare with the colonies of the fully virulent H37Rv culture in Fig. 3 *b*. × 125.

FIG. 7 *b*. BCG 317. Colonies on the surface of the agar medium containing 0.5 per cent albumin and 0.01 per cent Tween. The presence of a small amount of Tween in the medium has inhibited the formation of cords by this strain. The colonies are similar to those of the avirulent H37Ra strain in the presence of the same amount of Tween (see Fig. 2 *a*). × 125.

Published August 1, 1947



(Middlebrook *et. al.*: Virulence and morphology of mycobacteria)