GROWTH CHARACTERISTICS OF *MYCOBACTERIUM AVIUM* AND GROUP III NONPHOTOCHROMOGENIC MYCOBACTERIA IN HELA CELLS[1]

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ABSTRACT

BROSBE, EDWIN A. (Veterans Administration Hospital, Long Beach, Calif.), PAUL T. SUGIHARA, AND C. RICHARD SMITH. Growth characteristics of *Mycobacterium avium* and group III nonphotochromogenic mycobacteria in HeLa cells. J. Bacteriol. **84**:1282-1286. 1962.—The close relationship of *Mycobacterium avium* (avian) to group III nonphotochromogenic (Battey) strains of mycobacteria stimulated interest in their behavior in HeLa cells. In general, the avian strains grew more slowly than the Battey strains; 10 of 17 avian and 3 of 12 Battey strains required more than 7 days to exhibit abundant intracellular growth. Branching filaments were observed with variable frequency in all of the strains studied. Branching occurred at right angles, and was seen more often with the Battey strains and the avian strains isolated from swine. These observations indicate that the growth rate and growth pattern of avian and Battey mycobacteria in HeLa cells are not sufficiently specific to facilitate distinguishing the two species from each other.

Reports by Shepard (1957, 1958) and other investigators (Clark and Forrest, 1959; Brosbe, Sugihara, and Smith, 1961) indicated that cell culture techniques might be useful as an aid in classification of mycobacteria. The close relationship of *Mycobacterium avium* (avian) to group III nonphotochromogenic (Battey) strains of mycobacteria (Smith et al., 1960; Bojalil and Cerbón, 1960; Bönicke, 1960; Froman et al., 1961; Toda, Hagihara, and Takeya, 1961) stimulated interest in their behavior in HeLa cells. The growth characteristics of avian and Battey mycobacteria in HeLa cells are described in this paper.

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MATERIALS AND METHODS

Strains. Strains (14) of *M. avium* were furnished by S. Froman; 4 avian and 12 Battey strains were part of a "Batteypack" provided by E. Runyon. The source and the original investigator who sent each strain to Froman and Runyon are listed in Table 1. The avian strains were fully virulent for chickens (L. Scammon, *personal communication*).

Preparation of mycobacterial suspension. Suspensions were prepared from cultures 2 to 3 weeks old, depending on the extent of macroscopic growth. The method for preparing the bacterial suspension was described previously (Brosbe et al., 1961).

HeLa-cell cultures. HeLa cells were kindly supplied by D. Imagawa. The medium employed was composed of 60% basal yeast extract medium, 20% Scherer's maintenance solution, 10% Brain Heart Infusion Broth, and 10% calf serum (D. Imagawa, *personal communication*). The medium also contained 50 μg of streptomycin and 50 units of penicillin per ml. HeLa cells were harvested from stock cultures treated with 0.25% trypsin solution. Cells (60,000 to 75,000 in 1 ml of medium) were seeded on cover slips (11 by 22 mm) in Leighton tubes. The cultures were incubated for 48 hr at 37 C.

Inoculation of HeLa cells. Cell cultures were washed and inoculated as described in a previous report (Brosbe et al., 1961). Lamb serum replaced the calf serum in the yeast extract medium, to promote phagocytosis. Fetal bovine serum (Shepard, 1960) was used in later experiments.

Examination of cultures. The procedure of following intracellular growth has been described (Brosbe et al., 1961). The growth rate was estimated as moderate or rapid, based on the number of bacilli filling the cytoplasm or the extent of branching observed 5 to 7 days after inoculation.

TABLE 1. *Avian and Battey strains studied in HeLa cells*

Avian strain	Source*	Original investigator*	Battey strain	Source†	Original investigator†
F-61	Avian tuberculosis	A. Karlson	P-39	Pulmonary disease	C. E. Smith
F-63	Avian tuberculosis	A. Karlson	P-40	Pulmonary disease	F. Dunbar
F-66	Avian tuberculosis	A. Karlson	P-41	Pulmonary disease	F. Dunbar
F-540	Human gastric lavage	W. Feldman	P-42	Pulmonary disease	F. Dunbar
F-545	Avian tuberculosis	W. Feldman	P-44	Fatal systemic infection	S. Shephard
RH-8‡	Swine tuberculosis	S. Froman	P-45	Pulmonary disease	C. E. Smith
RH-17	Swine tuberculosis	S. Froman	P-46	Single sputum; no apparent disease	R. Gordon
RH-31	Swine tuberculosis	S. Froman	P-47	Fatal systemic infection	K. Ellis
RH-37	Swine tuberculosis	S. Froman	P-48	Fatal systemic infection	A. Karlson
RH-51	Swine tuberculosis	S. Froman	P-49	Fatal systemic infection	W. C. Yakovac
RH-53	Swine tuberculosis	S. Froman	P-54	Pulmonary disease	L. Affronti
RH-57	Swine tuberculosis	S. Froman	P-55	Pulmonary disease	H. C. Engbaek
RH-63	Swine tuberculosis	S. Froman			
RH-67	Swine tuberculosis	S. Froman			
P-50	Swine tuberculosis	S. Froman			
P-51‡	Swine tuberculosis	S. Froman			
P-52	Swine tuberculosis	S. Froman			
P-53	Swine tuberculosis	S. Froman			

* Personal communication from L. Scammon.

† Personal communication from E. Runyon.

‡ Same strain (L. Scammon, *personal communication*).

RESULTS

Intracellular growth rate. Most Battey strains grew more rapidly than did the avian strains (Table 2). No quantitative counts were done, however, and variable growth patterns (Fig. 1 and 2) undoubtedly influenced the accuracy of the estimate of intracellular growth rate. In general, strains which demonstrated good branching also appeared to show a faster growth rate.

Growth characteristics. All of the strains showed branching filaments with variable frequency. Branching seemed to occur at right angles (Fig. 3 and 4) and was observed more readily with the Battey strains and the avian strains recovered from swine (Table 3); 3 of the 17 avian and 2 of the 12 Battey strains exhibited a greater tendency for parallel alignment (Fig. 5 and 6). Occasionally, pleomorphic, club-shaped bacilli were seen in cell cultures infected with several strains of both species.

DISCUSSION

Although *M. avium* was not distinguished from the Battey bacillus employing HeLa cell cultures, we are inclined to agree with Durr, Smith, and Altman (1959) and Feldman (1961) that more evidence is needed to support the hypothesis that Battey strains are attenuated avian tubercle bacilli. The differential test of choice is virulence for chickens (Oatway, 1961). Growth-temperature (45 and 20 to 25 C) tolerance (Harris, 1960), specific tuberculins (Magnusson, Engebaek, and Bentzon, 1961), and arylsulfatase activity (Kubica and Beam, 1961) have been recommended as useful procedures for differentiating the two species.

Our findings were for the most part similar to those reported by Shepard (1958) and Clark and Forrest (1959). Their work, however, was concerned mainly with *M. kansasii* (Shepard, 1962) and the unclassified mycobacteria (Runyon, 1959). The one avian strain studied by Clark and Forrest (1959) resembled the growth characteristics of Battey bacilli.

Lateral branching was observed with some facility in the majority of Battey strains and in a number of avian strains, especially those isolated from swine. This growth pattern is of interest, since no filamentous forms and predominantly coccobacilli were seen in acid-fast-stained smears

TABLE 2. *Relative growth rates of avian and Battey mycobacteria in HeLa cells*

Strain	Intracellular growth rate	
	Rapid*	Moderate*
Avian	F-540	F-61
	P-50	F-63
	P-51†	F-66
	P-53	F-545
	RH-8†	P-52
	RH-31	RH-17
	RH-63	RH-37
	RH-67	RH-51
		RH-53
		RH-57
Battey	P-39	P-42
	P-40	P-46
	P-41	P-49
	P-44	
	P-45	
	P-47	
	P-48	
	P-54	
	P-55	

* See Materials and Methods section.

† Same strain (L. Scammon, *personal communication*).

of suspensions of both species prepared for inoculation of cell cultures.

The method used in this present work did not provide an accurate means of following the intracellular growth of avian and Battey mycobacteria in HeLa cells. Studies on the life cycle of mycelial strains of *M. avium* (Brieger and Fell, 1945; Brieger and Glauert, 1952) suggest that the frequency of finding branching filaments in a selected mycobacterial strain depends on the stage of the reproductive process. Also, the evidence for "true" branching by Battey and avian strains is considered inconclusive by some investigators (S. Froman, *personal communication*). Facilities for phase-contrast microscopy and time-lapse cinephotomicrography are therefore being developed in our laboratory, so that a better delineation of the morphology and behavior of the mycobacterial strain in an intracellular environment can be recorded.

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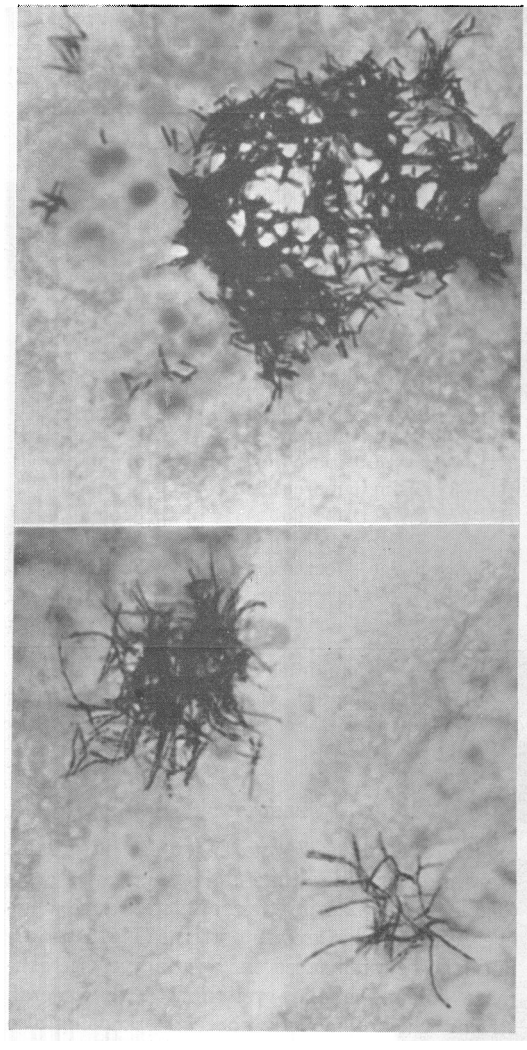
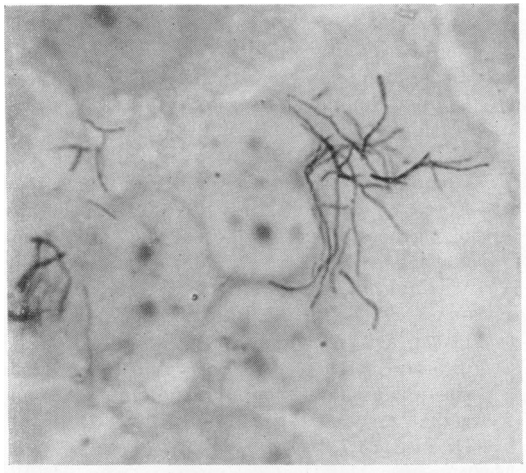


FIG. 1 (top). *Mycobacterium avium (F-540), 7 days after inoculation of HeLa cells. Growth rate as rapid as majority of Battey strains. Branching filaments were seen infrequently. Magnification* 1,200 ×.

FIG. 2 (bottom). *Battey strain (P-39) in HeLa-cell culture at 7 days. Abundant growth. Lateral branching readily observed. Magnification 1,100* ×.



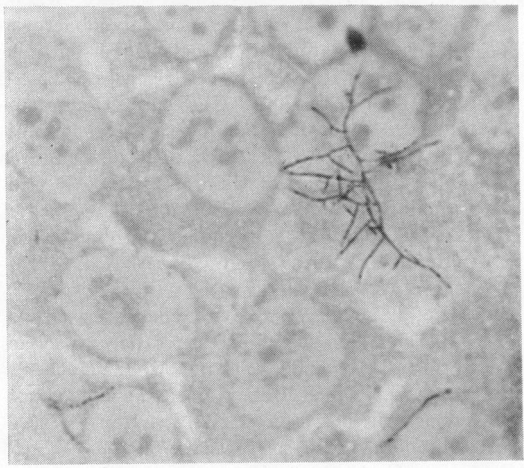


FIG. 3 (top). *Avian strain (RH-67), 5th day after inoculation. Growth pattern similar to that of many Battey strains. Magnification 1,200 ×.*

FIG. 4 (bottom). *Battey strain (P-40) at 3 days. Branching occurred for the most part at right angles. Magnification 1,100 ×.*

TABLE 3. *Frequency of branching exhibited by avian and Battey strains in HeLa-cell cultures*

Strain	Frequent*	Infrequent*
Avian	F-63	F-61
	P-50	F-66
	P-51†	F-540
	P-53	F-545
	RH-8†	P-52
	RH-31	RH-17
	RH-63	RH-37
	RH-67	RH-51
		RH-53
		RH-57
Battey	P-39	P-42
	P-40	P-46
	P-41	P-47
	P-44	
	P-45	
	P-48	
	P-49	
	P-54	
	P-55	

* Relative estimate based on ease of finding branching filaments in 3- to 7-day infected cell cultures.

† Same strain (L. Scammon, *personal communication*).

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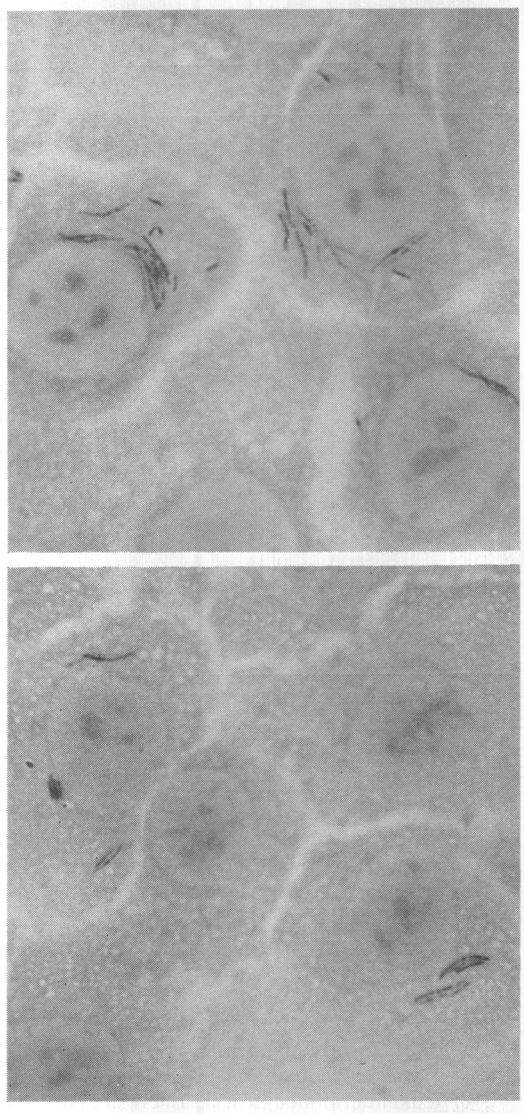

FIG. 5 (top). *Avian strain (RH-57), 4 days after inoculation. Tendency for bacilli to exhibit parallel alignment. Branching filaments were rare. Magnification 1,200 ×.*

FIG. 6 (bottom). *Battey strain (P-46) in HeLa cells, 4-day culture. Growth rate slower than most of the Battey strains. Infrequent branching with some suggestion of parallel alignment. Magnification 1,100 ×.*

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