Synonymy of *Mycobacterium simiae* Karasseva et al. 1965 and *Mycobacterium habana* Valdivia et al. 1971

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On the basis of their physiological and biochemical properties and their antigenic structures, as revealed by gel diffusion systems, *Mycobacterium simiae* Karasseva et al. 1965 and *Mycobacterium habana* Valdivia et al. 1971 are regarded as belonging to the same species. The correct name of this species, i.e., the legitimate name which has priority, is *M. simiae*. *M. habana* is thus a later subjective synonym of *M. simiae*.

Karasseva et al. (6) investigated spontaneous mycobacterial infections of monkeys and isolated numerous mycobacteria which differed in several properties from the known species of *Mycobacterium*. The isolates were identified as belonging to a new species, *Mycobacterium simiae*. *M. simiae* grows slowly and does not form pigment in the dark, but it has proved to be more or less photochromogenic. Representative strains caused severe lesions in mice. Further investigations (16) have shown that the isolates can be classified into two groups on the basis of biochemical and cultural properties and antigenic structure. The type strain of *M. simiae* (strain 29) was deposited in the American Type Culture Collection (14) and assigned the number 25275. Four strains were identified as *M. asiaticum* sp. nov. (17). Boisvert (1) of the Pasteur Institute isolated three strains of *M. simiae*: two from humans and one from a monkey. Both patients had been in Africa; one of them shed the bacterium in his sputum after having returned to France. Krasnow and Gross (8) reported on a case of *M. simiae* infection in the United States.

Valdivia et al. (13) isolated mycobacteria from sputum samples from 35 patients in Havana; the strains were identified as belonging to *M. habana*. In a colloquium held in Antwerp in 1972, Valdivia (12) presented 10 additional human cases yielding *M. habana*. The properties of *M. habana*, including its niacin-positive reaction, were investigated by Pattyn and Dommisse (10). It was shown that *M. habana* was weakly agglutinated by the antiserum of the Darden serotype of *M. intracellulare*. According to Boisvert, *M. habana* is identical with *M. simiae*. Meissner (9) examined seven strains of *M. simiae*, two strains of *M. asiaticum*, and three strains of *M. habana*. The *M. simiae* strains could be classified into two serotypes, namely, serotype I (strain 21) and serotype II (strain 59). The *M. habana* strains were agglu-

tinated, up to the dilution 1:60, by the antiserum to serotype I, and strains 20, 25, and 29 of *M. simiae* were agglutinated by the *M. habana* antiserum. Accordingly, *M. habana* proved to be identical with *M. simiae* serotype I. Kppler and Neubert (5) established that the biochemical properties and drug resistance spectra of *M. habana* and *M. simiae* are identical. They concluded that the two species belong to a single complex.

The investigation of the antigenic structure of mycobacteria by the gel-precipitation method has proved to be a very reliable method for demonstrating common antigenic components in different strains and for establishing the degree of relationship between strains (7, 15, 16). The identities of strains can be demonstrated by cross-absorption of the common antigens, and monospecific sera can be obtained by absorption with heterologous antigens. Based on these experiments, it seemed justified to apply the gel diffusion method to a comparative antigen analysis of *M. simiae* and *M. habana*.

MATERIALS AND METHODS

Bacterial strains. The type strain of *M. simiae* (strain 29 = ATCC 25275) was used to prepare antiserum in sheep (14). The antigenic structure of each of the following strains was examined: *M. simiae* 29 (ATCC 25275), *M. habana* 337, *M. habana* 4238 obtained from M. Kubin, Prague, *M. avium* ATCC 15768, *M. kansasii* ATCC 12478, and *M. smegmatis* ATCC 14468, the latter three obtained from the American Type Culture Collection, Rockville, Md.

Methods. The strains were grown in liquid Sauton medium. The harvested pellicle was collected on filter paper, washed with 0.9% NaCl solution, stirred with silicium carbide, extracted by cold (4°C) saline, and centrifuged at 4,000 rpm for 15 min. The supernatant was used as the cell-free antigen. It was standardized, with bovine serum protein as the standard, to contain 1% protein. Serum absorption and gel diffusion were performed as described previously (18).

In addition, some biochemical properties of *M. simiae* and *M. habana* were determined. The biochemical methods used were described previously by Bönicke (2), Käßler (4), and Wayne et al. (14).

TABLE 1. Biochemical properties of *M. simiae* strain 29 and two strains of *M. habana*

Test	<i>M. simiae</i> strain 29	<i>M. habana</i> strains 337 and 4238
Photoreactivity	+	+
Niacine	+	+
Phosphatase	—	—
Nitrate reductase	—	—
Urease	+	+
Nicotinamidase	+	+
Pyrazinamidase	+	+

RESULTS

The main biochemical characteristics of the type strain of *M. simiae* and the two strains of *M. habana* are shown in Table 1. The biochemical properties of 13 other strains of *M. simiae* were published in another paper (16). Strains of both species proved to be weakly photoreactive and niacin positive. Their enzyme reactions were identical.

The results of the immunodiffusion reactions are summarized in Table 2 and illustrated in Fig. 1 to 3. The anti-*M. simiae* serum formed 12 to 16 precipitation lines by titration with the homologous *M. simiae* antigen and the *M. habana* antigens as well. The lines of the homologous *M. simiae* system could be demonstrated

TABLE 2. Number of precipitation lines in gel diffusion reactions of *M. simiae* and *M. habana* antigens with unabsorbed *M. simiae* antiserum and the same antiserum after absorption by *M. habana* antigen and consecutively by *M. kansasii*, *M. avium*, and *M. smegmatis* antigens

Immunoserum	No. of precipitation lines					
	<i>M. simiae</i> 29	<i>M. habana</i> 337 ^a	<i>M. habana</i> 4238 ^a	<i>M. kansasii</i> ATCC 12478	<i>M. avium</i> ATCC 15768	<i>M. smegmatis</i> ATCC 14468
<i>M. simiae</i> 29 (not absorbed)	16	16	16			
<i>M. simiae</i> 29 absorbed by <i>M. habana</i>	4	4	4			
<i>M. simiae</i> 29 absorbed by <i>M. kansasii</i> + <i>M. avium</i> + <i>M. smegmatis</i>	4	4	4	0	0	0

^a Strains from M. Kubín, Prague, Czechoslovakia.

FIG. 1. Immunodiffusion reaction between *M. simiae* strain 29 antiserum (central well = M29) and antigens of *M. simiae* (sim 29), *M. habana* strain 337 (hab V), and *M. habana* strain 4238 (habR) antigens.

in the *simiae-habana* system (Fig. 1), thus indicating that the two organisms are identical.

Figure 2 shows the reaction of the anti-*simiae* serum absorbed by the antigen of *M. habana*. Since absorption with this antigen was not complete, the residual components had to be identified. The same four precipitation lines appeared in both the *M. simiae* and *M. habana* systems, indicating that no antigen absent in *M. habana* could be demonstrated in *M. simiae*. The position and intensity of the precipitation lines show that some antigenic components are in lower concentration in the *M. simiae* antigen than in the *M. habana* antigens.

The anti-*simiae* serum, which was absorbed stepwise by *M. avium*, *M. kansasii*, and *M. smegmatis* antigens, may be regarded as monospecific for *M. simiae*.

Figure 3 shows common precipitation lines between the *M. simiae* monospecific system and the *M. habana* antigens. Accordingly, the antigenic structures of *M. simiae* and *M. habana* proved to be identical in all three gel precipitation systems.

DISCUSSION

Boisvert (1) and Meissner (9) have shown that *M. habana* is not a distinct species of *Mycobacterium*, for it is identical with *M. simiae*, a species described in 1965 and thoroughly examined and characterized thereafter. Pattyn (10), on the other hand, is of the opposite opin-

ion. A direct comparison of the antigenic structure of the two organisms seems to be the most reliable method of answering this question.

We have shown that the 16 antigens demonstrated by us in *M. simiae* are present in *M. habana* as well. Therefore, in our opinion, the latter does not represent a new species, and this is confirmed by the biochemical reactions of the organisms. However, the biochemical tests have not been standardized, and this may explain why *M. simiae* strain 29 was found to be niacin negative and nitrate reductase positive by Pattyn and niacin positive and nitrate reductase negative by Boisvert (1), Kppler (5), Schrder (11), Meissner (9), and by us (15-17). *M. habana*, which we regard as a later subjective synonym of *M. simiae*, deserves interest as a human pathogen, especially in tropical countries. We believe that it is not by mere chance that this species has been isolated from humans in tropical countries, namely, Africa and Cuba: hot, humid climate favors its multiplication in nature. It is of special importance (9) that two serotypes of *M. simiae* could be differentiated: serotype I (strain 29) and serotype II (strains 55, 58, and 59). Earlier, we (15, 17) observed some differences among strains identified by us as *M. simiae*, but we attributed these divergencies to strain variability. A relationship between *M. simiae* and the "*avium-intracellulare*" group is suggested by the eight common antigens demonstrable by the gel precipitation

FIG. 2. Immunodiffusion reaction between *M. simiae* antiserum absorbed by the antigen of *M. habana* (29/hab) and the antigens of *M. simiae* (sim 29) and *M. habana* (hab R and hab V).

FIG. 3. Immunodiffusion reaction between *M. simiae* strain 29 antiserum absorbed by *M. avium* + *M. kansasii* + *M. smegmatis* (29/laks) antigens and the *M. simiae* (sim 29), *M. habanna* (hab R and hab V), *M. kansasii* (k), *M. avium* (a), and *M. smegmatis* (s) antigens.

test and by the cross-agglutination reactions at low titer, demonstrated by Pattyn (10) and Meissner and Schröder (9). We (16) have also noted this antigenic relationship.

REPRINT REQUESTS

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