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The 47th Annual Meeting Invitation Lecture

MYCOBACTERIA AND MYCOBACTERIOSES*

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It is an inspiration to be here with you, who, in spite of vastly different origin and background, share with me understanding of and interest in mycobacteria and mycobacterial diseases. It was Goethe who said, "To know someone here or there, with whom you can feel there is understanding in spite of distance or thoughts unexpressed-that can make of this earth a garden". It is good to know that in spite of our difficulties with language we share much in our interests and experience. Let us continue to perfect a garden of understanding, with your beautiful bridges, bridges of understanding, shared emotions, objectives, connecting our distant countries.

For understanding we require communication. In the arched bridges and curving streams characteristic of your beautiful Japanese gardens I see symbols of communication. But given good communication, DIFFERENCES we should *maintain*. Japanese should remain Japanese, Englishmen remain English, Blacks should maintain their black heritage. Of course as an American, I think all Japanese should learn English; but long live Japanese bridges, Shinto temples, kimonos, geishas, kabuki!

Bacteriological nomenclature has no nationality. It is international, and *should* be. Only one name should be assigned and used for a given kind of bacterium. This name is not determined by the United States of America, not by the Head of some Bacteriology Department, not by the Chief of an Institute, not even by the name printed on the label of the culture. The right name is the one, the only one, which is in accord with the International Code of Bacterial Nomenclature: the first name validly published, corresponding to a described and designated *type strain*, cultures of which are preserved in and available from a national type culture collection. This is our heritage. But times are changing. The Code needs revision, and it is being revised. Compliance to the International Rules does not establish *adoption* of a species name.

Species name, like any other name, must be *used* to become established. Common use of a species name will usually follow demonstration, preferably in laboratories other than that of the name's author, that the taxon described is really distinctive. There are probably more than 250 *Mycobacterium* species names in the literature which should not be used. The International Subcommittee for *Mycobacterium*, as established by the International Committee on Systematic Bacteriology of the International Association of Microbiological Societies, is seeking to prepare a list of species names which they consider legitimate and inclusive of all the well-described mycobacteria of the earth. Assistance in this project is sought from all worthy sources. Names not on the list when finally prepared would then appropriately be discarded. Of course, what we require, to judge the worthiness of a new species proposal, is thorough comparison with strains of previously named species. Forty-four mycobacterial species names appearing in the current literature were submitted to the *Mycobacterium* Subcommittee for their expression of opinion as to legitimacy and the possible distinctiveness of the indicated taxa. Only 26 names were considered to be of reasonably well-studied taxa. Not all of these were fully acceptable to this subcommittee. Only nine names received unanimous acceptance, five were accepted by only about 60%, and

Mycobacterium Species Names

	A	R	D
M. avium Chester, 1901	11		
M. bovis Karlson & Lessel, 1970	11		
M. chelonei Bergey *et al.*, 1923	9	1	1
M. diernhoferi Bönicke & Juhasz,	6	5	
M. flavescens Bojalil *et al.*, 1962	8	3	
M. fortuitum da Costa Cruz, 1938	9	1	1
M. gastri Wayne, 1966	9	2	
M. gordonae Bojalil *et al.*, 1962	9	2	
M. intracellulare (Cuttino & McCabe)	7	4	
M. kansasii Hauduroy, 1955	11		
M. leprae (Hansen) L & N, 1896	11		
M. lepraemurium Marchoux & Sorel,	10	1	
M. marinum Aronson, 1926	11		
M. microti Reed, 1957	9	2	
M. nonchromogenicum Tsukamura, 1965	7	4	
M. paratuberculosis Bergey *et al.*,	9	2	
M. phlei L & N, 1899	11		
M. scrofulaceum Prissick & Masson,	10		1
M. smegmatis (Trevisan) L & N, 1899	11		
M. terrae Wayne, 1966	7	3	1
M. thamnopheos Aronson, 1929	4	7	
M. triviale Kubica, 1970	5	6	
M. tuberculosis (Zopf) L & N, 1896	11		
M. ulcerans MacCallum, 1950	9	2	
M. vaccae Bönicke & Juhasz, 1964	7	4	
M. xenopi Schwabacher, 1959	11		

Subcommittee opinion, 1971
A=accept R=reserve judgment D=discard

Common Errors in Nomenclature

Incorrect	Correct
M. tuberculosis var. *hominis*	*M. tuberculosis*
M. tuberculosis var. *bovis*	*M. bovis*
M. "aquae"	*M. gordonae*
M. abscessus	*M. chelonei*
M. xenopei	*M. xenopi*
M. balnei	*M. marinum*
M. tuberculosis (BCG)	*M. bovis* (BCG)
"Strain", meaning species	Species
"Atypical"	Other mycobacteria, or use species names or Group
"Saprophytes", implying exclusion of such species as *M. gordonae*, *M. fortuitum*	Use species names

two (*thamnopheos* and *triviale*) were approved by less than half the voting members.

In the interests of consistency and precise communication, one should avoid some rather common errors.

The different species of mycobacteria are quite heterogenous in some respects but share many properties. Most species can live saprophytically. In our experience the most common saprophytes in clinical specimens are not those mentioned in the textbooks, *M. phlei* and *M. smegmatis*. We do not find *M. smegmatis* to occur, as is often implied, in urine or other clinical specimens. The common saprophytes do include some rapid growers, usually *M. fortuitum*, but in many areas slow growers of Groups II and III are the most commonly encountered saprophytes. It is possible—even probable—that the potential pathogens of these Groups may also exist saprophytically in nature.

We are interested in mycobacteria. I know *you* are interested in mycobacteria because you have published so many outstanding papers concerning these organisms. Your contributions have been greatly important and cover every aspect. I cannot speak too highly of your most excellent investigations. If we attain the objectives which you are seeking, the future of tuberculosis control is bright.

We are interested in the mycobacteria-and in man. Mycobacteria, Protista obviously of the simplest structure and minute in size, at first glance appear to be remotely removed in nature from man. We are animals belonging to the genus *Homo*. But mycobacteria and man have much in common. Protoplasms of man and mycobacteria, although somewhat differently organized, have similar elementary composition. Mycobacterial cells of several species, like our own cells, find in our bodies conditions of temperature, water, oxygen, pH and nutrients which are most favorable. This similarity of protoplasms is largely responsible for the very slow development of drugs effective in treatment of tuberculosis. What is injurious to tubercle bacilli is usually injurious to man. *Mycobacterium leprae*, *Mycobacterium tuberculosis* and some other mycobacterial species are exclusively devoted to people, survive for very long periods in contact with our tissues. People are beautiful; so are mycobacteria. Mycobacteria may eventually kill people. I am sad to say, so do people kill people.

Most of you, I presume, are clinicians, medical practitioners. I am a microbiologist. I strongly urge your close acquaintance and collaboration with microbiologists who can show you the growing mycobacterial pathogens of your patients and help you interpret laboratory results. Conditions in test

tube or guinea pig are just possibly a little different from those in your patients' lungs. But the record is repeatedly seen that the best patient care, the best control of patients' disease is where clinician and microbiologist closely collaborate. A bonus for the clinician is some contact with the fascinating, living microbes. Each one-like each person-is different. Microbe peculiarities are best seen by examination of plate cultures.

If we wish to know something about mycobacteria, we should look at them. They exist as individual cells for which the highest power microscopes are needed. Mycobacteria also form colonies. These are not just piles of bacilli. They have organizations which often are very distinctive, exceedingly interesting, even beautiful. For many years, culture of tubercle bacilli was almost wholly on opaque media, usually containing eggs. The introduction of transparent agar media, such as the Middlebrook-Cohn 7 H 10 medium, and the use of plastic Petri dishes which have perfectly flat, thin tops and bottoms has facilitated the examination of growth with comparatively low (100 ×) magnification. Petri plates are placed inverted on the stage of the microscope. In this way cellular and colonial growth and organization can readily be studied. This proves to be of great assistance in identification of mycobacterial pathogens. Shortly, I will outline a few of the prominent colonial and other distinctive characteristics we have found most useful for identification of mycobacterial pathogens. More important, microscopic examination of plates provides for *early* reporting. The long delay in reporting the presence or absence of tubercl bacilli in a sputum, or in reporting drug susceptibilities has always been annoying to the medical staff and sometimes even dangerous to the patient. While determination of growth by naked eye requires a matter of *weeks*, by use of microscope it may be accomplished in *days*.

We can confidently expect even in the near future much earlier reporting of tests for tuberculosis and drug susceptibilities. Less probable in the near future, but even more important, would be a drug treatment program leading to more rapid sterilization of tuberculous lesions, change from one, two or more *years* of drug treatment to only a few *weeks*. This is an objective diligently to be sought.

Have mycobacteria recently evolved? We believe that mycobacteria and mycobacterioses of former years were much the same as they are now. Mycobacteria may have changed somewhat, but man's understanding of them has charged more. Some species of mycobacteria probably originated more recently than others. However, there is no evidence indicating that *M. kansasii*, *M. marinum* or *M. scrofulaceum*, for example, have been recently evolved, as, perhaps, from tubercle bacilli. The concept of what tubercle bacilli are has undergone evolution; and the disease tuberculosis has become better defined. For example, superficial lesions caused by *M. marinum* were no doubt thought to be tuberculous. These acid-fast bacilli were noted to be difficult to grow, and this is certainly what is to be expected for *M. marinum*, unless incubation is at lower-than-usual temperature. Diseases such as swimming pool granuloma, Buruli ulcer, infections due to *M. kansasii*, *M. intracellulare* and some other recently recognized mycobacteria *probably* have been with us, like tuberculosis, for centuries; but all of these in the past, insofar as they were recognized at all, were thought to illustrate various pleomorphic aspects of tuberculosis. It must be admitted that this simple explanation is not fully adequate. It was only seven years ago that Marks and Schwabacher called attention to *M. xenopi* as a pathogen for man. This species, described first as a pathogen of a toad, is now in some areas second only to tubercle bacilli in frequency of occurrence in sputum and other clinical specimens. *M. xenopi* is so completely distinct, it is hard to understand how it could have remained wholly unnoticed for so many decades. I predict that you will find *M. xenopi* in Japan.

Leprosy bacilli through the years have retained their uniqueness. Currently, there has been challenge to their classification as mycobacteria. This is unwarranted. Leprosy bacilli are distinctive in losing acid-fastness on extraction with pyridine; but homology in immunology, and in electron microscopically-revealed structure confirms their retention in the genus *Mycobacterium*.

We come now to practical matters of mycobacterial identification. How long does it take to receive a report on a submitted sputum: "Culture positive for *M. tuberculosis*"? Commonly, it is four or five

weeks. Negative reports are properly even slower to arrive. By use of microscope examination of plate cultures, at least a provisional report may be obtained, often in less than one week (Lantern slide). Colonies of tubercle bacilli usually have a prominently patterned texture due to tight cording of the bacilli. Colonies soon become opaque. Smooth colonies are never seen. Of course, other properties must be known before *M. tuberculosis* can be definitely identified: slow growth, a positive niacin test, and at least one of the following: (1) nitrate reduction, (2) negative catalase after heating at 68°C, and (3) typical drug susceptibility.

Tubercle bacilli of the past certainly had the same characteristics as we know them to have today. Koch recognized the typical serpentine stranding of tubercle bacilli. Moreover, *M. bovis* was recognized as distinct from *M. tuberculosis* before 1900. Today we have in addition to these two species of tubercle bacilli, *M. microti*, which has a different pattern of pathogenicity including virulence for voles ; and *M. africanum*, a newly recognized species pathogenic for man, and occurring principally in parts of Africa.

Patients sometimes have tuberculosis-like symptoms but not true tuberculosis. From their sputum grow colonies of acid-fast bacilli which are vastly different from colonies of tubercle bacilli. Any of several different species may be involved, each having characteristic colonies.

Colonies may be so thin and transparent as to be almost invisible macroscopically. *M. intracellulare* and *M. avium* in primary culture are of this nature (Lantern slides). Disease due to these agents has been recognized on all continents, is particularly frequent in certain low altitude, warm temperature areas, as in our southeastern states and in western and northern Australia. I would expect a greater incidence of Battey disease in rural Kyushu and southern Honshu than in Hokkaido. It is of little or no clinical importance if a strain is *M. avium* or *M. intracellulare*. In addition to recognizing the typical colonies, demonstration of negative Tween-hydrolysis is all that is required to identify this avium-Battey complex. Saprophytic strains are regularly Tween positive, while potential pathogens are negative. Commonly, a small proportion of the colonies of a patient strain may be more or less rough and rarely all of the colonies will be rough. They will be immediately recognized as not *M. tuberculosis* by negative niacin, negative nitrate reduction, positive catalase after heating at 68° and drug resistance.

In addition to tuberculosis-like pulmonary disease, strains of the avium complex may be the cause of cervical lymphadenitis, and more rarely of generalized disseminated disease including osteomyelitis. An early report of this type of disease was that of Cuttino and McCabe:

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PURE GRANULOMATOUS NOCARDIOSIS. A NEW FUNGUS DISEASE DISTINGUISHED BY INTRACELLULAR PARASITISM

A DESCRIPTION OF A NEW DISEASE IN MAN DUE TO A HITHERTO UNDESCRIBED ORGANISM, NOCARDIA INTRACELLULARIS, N. SP., INCLUDINC A STUDY OF THE BIOLOGIC AND PATHOGENIC PROPERTIES OF THIS SPECIES*

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All authorities who have studied this strain agree that it is not of a *Nocardia* species but a *Mycobacterium*: *M. intracellulare*. This name was the first one to be published for the taxon referred to as "Battey bacilli". Of course, these are not fungi, and Nocardiae are not fungi either. A summary of reports of disseminated disease due to the avium complex is seen on the next slide (Table 3). Most of the cases were infants or children; a majority failed to recover or relapsed. Inclusion of the second case of Krieger *et al.*, which was thought to be caused by a Group II scotochromogen, may seem to be in error. But Marks, using whole-cell lipid extract analyses and Schaefer, studying agglutination, find

Disseminated Disease Due to *M. Avium* Complex (Modified from Davis *et al.*)

Authors	Age at Onset	Sex	Outcome	Comments
Cuttino and McCabe, 1949	34 months	F	Died-4+ months	Granulomatous intracellular parasitism
Weed et al., 1956	6 years	M	Improved, short followup	Ten year history. Recurrent draining sinuses
Van der Hoeven et al., 1958	19 months	M	Died-34 months	Extensive visceral disease
Yakovac et al., 1961	19 months	M	Died-21 months	Extensive visceral disease
Krieger et al., 1964	5 years	M	Improved-relapsed, 2 years	Recurrent
	10 months	F	Recovered	Pulm. & osteomyelitis; Group II
Volini et al., 1965	2 months	M	Died-42 months	Extensive visceral disease
Davis et al., 1966	19 years	M	Improved-relapsed	"Good response to chemotherapy"

no boundaries between strains of Group II and the avium complex. We find them quite distinct in allergens, the stimulation of specific skin hypersensitivity in guinea pigs.

This brings us again to a consideration of bacterial names. Species do not occur in nature. They are an invention of man, needed for communication, record. But if species intergrade conspicuously, have prominent shared antigens, of what value is the species name? If we need to be precisely definite, we must use serotype or other descriptive and distinctive designations. These are important in epidemiological investigations. The serotypes of the avium-intracellulare complex which Schaefer has found in human patients are shown on the next slide.

Note that although these strains from human tuberculosis-like lesions are regularly considered to be Battey bacilli (*M. intracellulare*) the two principal *M. avium* serotypes, I and II, are included. Strains of serotype Avium I from birds are usually pathogenic for birds, but from man they usually are not. We sent Schaefer 20 strains which were recent isolates from patients at Battey State Hospital (Georgia, U.S.A.), the hospital from which Battey bacilli got their name (Lantern slide).

Battey-Avian Strains from Man
Serotyped by Schaefer, 1967

Serotype	No. of strains	Serotype	No. of strains
VII/Howell	44	Darden	12
Boone	23	Scrof/Arnold	8
Yandle	21	VI	7
Watson	17	Altman	7
Davis	14	Avium II	6
IV	14	III/IV	3
Avium I	13		

Serotypes of 20 Patient-strains
from Battey State Hospital, Rome,
Georgia by Schaefer, 1971

Boone	3	Davis	2
Yandle	3	Dent	1
Avium I	3	Lunning	1
Howell	2	Unclas.	1
VII	2	Spon. Agglut.	2

Much to our surprise three of these "Battey strains" were serotyped Avium I. Shall we conclude that Battey bacilli include strains of *M. avium*? We should. The expression "*M. avium* complex" emerges as appropriate. This also reinforces the decision that species designation here is of very much less value than serotype. It is of interest to note here also that one of these serotypes is Lunning. Most strains of Lunning serotype are scotochromogens (species *M. scrofulaceum*). On the previous slide, we saw also that Schaefer includes as one serotype "Scrof/Arnold." We conclude that some strains of *M. intracellulare* and of *M. scrofulaceum* have common agglutininogens. However, as we shall see, such strains have distinctive allergens. Clearly, we need more information concerning correlation of serological and other properties before species boundaries between *M. avium*, *M. intracellulare* and perhaps *M. scrofulaceum* can be described. By collaborative studies we hope to establish

whether bacillary agglutination serotypes are consistent with other serotypes as by fluorescent antibody, Ouchterlony precipitin tests and other properties such as cell wall lipid analyses. Until such information is available, species names in this area are of indefinite meaning. The designation "*M. avium* complex" is better.

M. scrofulaceum (Group II). These are strains which are pigmented at all stages of growth and on all media irrespective of exposure to light. No colony characteristics reliably distinguish scotochromogenic strains which at times are pathogenic (*M. scrofulaceum*) from those which only very rarely are (*M. gordonae*). These two species are most readily distinguished by the Tween hydrolysis test, this again being negative for the potential pathogen, *M. scrofulaceum*. *M. gordonae* is different also in lacking amidase activity or showing only urease activity (some strains). Both *M. scrofulaceum* and *M. gordonae* may occur in water, but occurrence as pathogens in man is well established only for *M. scrofulaceum*.

M. xenopi. Very slowly developing colonies that regularly have a fringe of filaments in early growth on 7 H 10, or persistently on cornmeal agar, are a very distinctive feature of this species. Colonies are small and, at maturity, usually yellow. Cells of this species are quite diagnostic: they are long and thin. No growth occurs at 25°C, nor in media containing isoniazid or streptomycin, 1～2 mcg/m*l*. Rarely, *M. xenopi* lacks yellow color. If nonpigmented colonies with branching filamentous extensions appear, these may be *M. fortuitum*, which on primary culture sometimes does not exhibit the characteristic rapid growth. Other tests mentioned above must be made. *M. fortuitum* growth readily at 25°C, and is resistant to 5 mcg/m*l* isoniazid.

M. kansasii. *M. kansasii* is universally recognized by yellow pigmentation, which develops only if the growing cultures are exposed to light. If plates are incubated in continuous light, *M. kansasii*, forms crystals of carotene, and the appearance of these on or in colonies from a specimen source other than a superficial body area (as sputum) is alone enough for almost certain identification of *M. kansasii*.

Colony centers are thickened (darker); the thinner peripheral portions show more or less stranding of bacilli. Strains vary in roughness, commonly are intermediate (SR.....RS), but may be completely rough or, rarely, fully smooth. As seen by reflected light (stereomicroscope), the colonies have a characteristic sheen produced by a discontinuous surface (waxy?) film. This distinctive feature is present irrespective of whether the *M. kansasii* colonies are pigmented. Growth on cornmeal agar is very poor or lacking.

Occurrence of nonphotochromogenic *M. kansasii* is extremely rare. Scotochromogenic strains are distinctive in producing exceedingly abundant crystals of carotene, an appearance never seen on the usual Group II strains. Nonpigmented *M. kansasii* strains have the typical colony morphology of the species and, in contradistinction to *M. gastri* (which has somewhat similar colonies), reduce nitrate and have active catalase after heating at 68°C. (Lantern slides)

M. marinum. Colonies are similar to those of *M. kansasii*, but usually they are smoother and rhizodes are more readily seen. Distinguishing features: (1) source never in sputum; always from superficial lesion; (2) at 37°C poor or no growth on initial isolation; at 25°C growth is much more rapid than that of *M. kansasii*; (3) nitrate not reduced.

M. ulcerans. *M. ulcerans* is recognized by its source (superficial lesion on person from tropical area), growth restriction to 32 to 33°C, and by *very* slow growth (often two months or more). Niacin test may be weakly positive; colonies show serpentine stranding.

M. fortuitum, *M. chelonei*. Occasionally a strain of one of the species of rapidly-growing bacteria does not grow rapidly on primary isolation. Other properties such as colonial appearance may help in identification; mutation in subcultures will soon result in the characteristic rapid growth. Species delineation in this area is far from settled. Two species or species-complexes are recognized among the arylsulfatase-positive, potential pathogens: *M. fortuitum* and *M. chelonei*. *M. fortuitum* regularly produces mycelial colonies on either 7 H 10 or cornmeal agar in 24 hours. *M. chelonei* does not. Fully mature

colonies are seen after 3 or 4 days. On cornmeal agar many colonies of *M. fortuitum* will be found still to have branching filamentous extensions, some of these on the agar surface, others penetrating into the medium as a kind of substrate mycelium. On 7 H 10 or other rich media, mature colonies ordinarily do not show filamentous extensions, but these may be seen where colony growth has been checked by crowding. A few other species of rapid growers have similar colonies (e.g., *M. smegmatis*), but these species almost never appear in clinical specimens, and are distinct in being arylsulfatase-negative.

M. chelonei, whether of smooth or of rough colony type, is distinctive in lacking branching filamentous extensions. The colonies are thin, with a fine granular texture. On 7 H 10 the colonies are circular in outline, flat at first, then become low domed. On cornmeal agar smooth colonies usually are radially lobed. Rhizodes, conspicuous on *M. fortuitum*, are absent or inconspicuous on *M. chelonei*.

Let us cultivate a world garden with harmony encompassing diversity. May our communication be kept accurate, precise, by adherance to internationally accepted nomenclature. Great strides in control of tuberculosis will occur if an immunogen better than BCG in being stable, nonallergenic is obtained and used; and if a test indicating need of vaccination is devised; and if a drug or drug regimen can be found which will act promptly, not requiring years of therapy. Finally, it is my conviction that your own future, our future, will be much brightened by closer collaboration of clinician and microbiologist, by utilization of methods here described by which the living, growing mycobacteria are seen to display their fascinating characteristics.