

## The Immunological and Epidemiological Significance of Environmental Mycobacteria on Leprosy and Tuberculosis Control<sup>1</sup>

Leprosy and tuberculosis together must rate as among the world's most common disabling diseases. Current estimates suggest that around 50 million people have or recently have had tuberculosis<sup>2</sup> and upwards of 12 million suffer from leprosy<sup>3</sup>. Although both diseases were once common in northwestern Europe, they are now comparative rarities in the region, being on the whole confined to immigrants and the elderly. They are now most common in the developing countries where, because of their chronic and debilitating nature, both diseases make extensive demands on the limited medical resources of such countries. Thus, the control and eventual eradication of tuberculosis and leprosy is extremely important for the future health and wealth of the world's poorer nations.

The attempts to control both diseases fall into two categories: a) those aimed at early identification and treatment of cases, particularly infectious cases, and b) those aimed at preventing disease in susceptible individuals by vaccination or chemoprophylaxis. Vaccination with BCG has been used extensively in the prevention of tuberculosis, both in the developed and developing world, and much hope has been attached to the possibility of its use for the prevention of leprosy. However, results of controlled trials of the effectiveness of BCG vaccination against both diseases have shown varying degrees of protection in the past<sup>4</sup>, with the

medical world being most recently shocked by the results of a trial in South India<sup>5</sup> which showed that BCG apparently afforded no protection whatsoever against tuberculosis in the population tested.

Many possible reasons for the variability in the efficacy of BCG have been put forward, with several investigators suggesting that immunological crossreactions of some sort between environmental mycobacteria and the leprosy and tubercle bacilli may be involved. Such crossreactivity may also interfere with the use of immunological tests in the diagnosis and epidemiological investigation of both leprosy and tuberculosis. On the other hand, this crossreactivity may possibly allow the production of better vaccines against the two diseases. It is these and related issues which are to be discussed in this essay together with a basic introduction to the environmental mycobacteria, their ecology, and their role in human infection.

### Environmental mycobacteria

**Definitions and classification.** The term "environmental mycobacteria," is used in this essay in line with current usage by several researchers<sup>6-9</sup> to refer to those mycobacteria which under natural conditions can reproduce in the nonmammalian external environment. This definition thus excludes both the human and bovine forms of the tubercle bacillus as well as the noncultivable obligate mammalian parasite, *Mycobacte-*

<sup>1</sup> This review was written in 1982 by Mark J. Pallen, B.A. (Hons.), Cantab., while a medical student at London Hospital Medical College. It was a prize-winning essay in the annual competition set up by the British Leprosy Relief Association (LEPRA) for essays on various aspects of leprosy. We take pleasure in publishing this review. Mr. Pallen's present address is 15 Thornaby House, Canrobert Street, London E2 0BE, England.

<sup>2</sup> Robbins, S. L. and Cotran, R. S. *Pathologic Basis of Disease*. 2nd ed. Philadelphia: W. B. Saunders Co., 1979, p. 397.

<sup>3</sup> Lincoln, E. M. and Gilbert, L. A. Disease in children due to mycobacteria other than *M. tuberculosis*. *Am. Rev. Respir. Dis.* **105** (1972) 683-716.

<sup>4</sup> Price, J. E. BCG vaccination in leprosy. *Int. J. Lepr.* **50** (1982) 205-212.

<sup>5</sup> Tuberculosis prevention trial, Madras. Trial of BCG vaccines in South India for tuberculosis prevention. *Indian J. Med. Res.* **72** Suppl. (1980) 1-74.

<sup>6</sup> Is BCG vaccination effective? *Tubercle* **62** (1981) 219-221.

<sup>7</sup> Rook, G. A. W., *et al.* The effect of two distinct forms of cell-mediated response to mycobacteria on the protective efficacy of BCG. *Tubercle* **62** (1981) 63-68.

<sup>8</sup> Stanford, J. L., *et al.* A preliminary study of the effect of contact with environmental mycobacteria on the pattern of sensitivity to a range of new tuberculin amongst Ugandan adults. *J. Hyg. (Camb.)* **76** (1976) 205-214.

<sup>9</sup> Stanford, J. L. A mycobacteriologist's view of the immunology of leprosy. *Bull. Inst. Pasteur* **79** (1981) 261-273.

TABLE 1. *Some important species of environmental mycobacteria with selected synonyms<sup>a</sup> as defined by immunodiffusion studies.*

Slow-growing mycobacteria	Fast-growing mycobacteria
<i>M. avium</i> ( <i>M. intracellulare</i> )	<i>M. chelonae</i> ( <i>M. abscessus</i> )
<i>M. gordonae</i>	<i>M. chitae</i>
<i>M. kansasii</i> ( <i>M. gastrii</i> )	<i>M. diernhoferi</i>
<i>M. marinum</i> ( <i>M. balnei</i> )	<i>M. duvalii</i>
<i>M. nonchronogenicum</i> ( <i>M. terrae</i> )	<i>M. flavescens</i>
<i>M. scrofulaceum</i> ( <i>M. marianum</i> )	<i>M. fortuitum</i>
<i>M. ulcerans</i> ( <i>M. buruli</i> )	<i>M. gilvum</i>
<i>M. xenopi</i>	<i>M. phlei</i>
	<i>M. rhodesiae</i>
	<i>M. smegmatis</i>
	<i>M. thermoresistibile</i>

<sup>a</sup> Synonyms are shown in parentheses. This table is based on Tables 1 and 2 in Stanford and Grange<sup>15</sup>.

*rium leprae*, even though bacteria from this species may, of course, remain viable for extensive periods when shed into the external environment by infectious individuals<sup>10</sup>. This definition must also, at least for the time being, exclude those species of mycobacteria, e.g., *M. szulgai*<sup>11</sup> and *M. lepraemurium*, which can only so far be primarily isolated from mammalian sources. In effect the term covers much of the same ground as the older designation "atypical mycobacteria," but in addition includes some nonpathogenic species of mycobacteria, e.g., *M. vaccae*.

Although both the leprosy and tubercle bacilli were first described over 100 years ago, over half of all known species of the environmental mycobacteria have been recognized in the last 30 years. *Bergey's Manual of Determinative Bacteriology*<sup>12</sup> (eighth edition) lists some 25 species of mycobacteria and at least two dozen or so other mycobacterial taxa have been proposed as meriting species status<sup>12</sup>. Members of the genus *Mycobacterium* may be recognized<sup>12</sup> as nonmotile, nonsporulating, pleomorphic,

Gram-positive bacilli, exhibiting surface, rope-like, peptidoglycolipid structures and the specific property of mycobacterial acid fastness, that is, resistance to decolorization by acid alcohol after staining with certain arylmethane dyes. The genus *Mycobacterium* is closely related to the taxa *Corynebacterium* and *Nocardia*, and some<sup>12</sup> refer to all three groups together as the CMN group of microorganisms. Within the genus *Mycobacterium*, species may be defined by both biological criteria<sup>13</sup>, in particular by numerical taxonomic methods<sup>14</sup>, and by immunodiffusion analysis<sup>15</sup>. Both methods show a distinction at the subgeneric level into fast-growing and slow-growing species. Fast-growing species exhibit growth on Lowenstein-Jensen medium within five days at 37°; whereas slow-growing species require more time to show growth under such conditions.

Immunodiffusion analysis<sup>15</sup> has shown that among the strains tested, all mycobacteria possess three types of antigens: 1) antigens common to all mycobacteria (group i antigens), 2) antigens common to either the slow-growing species (group ii antigens) or the fast-growing species (group iii antigens), and 3) species-specific antigens (group iv antigens). Table 1 lists some 19 species of environmental mycobacteria, as defined by immunodiffusion studies, with some important synonyms.

**Ecology of the environmental mycobacteria.** While the ultrastructure, biochemistry, genetics, and antigenic structure of the environmental mycobacteria have all been investigated extensively, relatively little is known about the habitats occupied by these microorganisms and their geographical distribution, both on a worldwide and on a local scale. Environmental mycobacteria of one sort or another have been isolated from a wide variety of inanimate sources:

<sup>10</sup> Desikan, K. V. Viability of *M. leprae* outside the human body. *Lepr. Rev.* **48** (1977) 231-235.

<sup>11</sup> Schaffer, W. B., et al. *M. szulgai*—a new pathogen, serologic identification and report of five new cases. *Am. Rev. Respir. Dis.* **108** (1973) 1320-1326.

<sup>12</sup> Buchanan, R. E. and Gibbons, N. E. *Bergey's Manual of Determinative Bacteriology*. 8th ed. Baltimore: Williams and Wilkins Co., 1974.

<sup>13</sup> Kubica, G. P. Differential identification of mycobacteria. VII. Key features of identification of clinically significant mycobacteria. *Am. Rev. Respir. Dis.* **107** (1973) 9-21.

<sup>14</sup> Wayne, L. G. Numerical taxonomy and cooperative studies: roles and limits. *Rev. Infect. Dis.* **3** (1981) 822-828.

<sup>15</sup> Stanford, J. L. and Grange, J. M. The meaning and structure of species as applied to mycobacteria. *Tubercle* **55** (1974) 143-152.

soil<sup>8, 16-21</sup>, vegetables<sup>22</sup> (including a radish, the original source<sup>23</sup> of the so-called "radish bacillus," *M. terrae*); house dust<sup>7, 24</sup>; operating theater sinks<sup>25</sup>; and tap<sup>26-28</sup>, river<sup>29</sup>, pond<sup>17</sup>, and coastal<sup>30</sup> water. In addition, several species of mycobacteria are pathogenic to, and have been isolated from, non-mammalian vertebrates, including species of birds<sup>31</sup>, reptiles<sup>32</sup>, amphibians<sup>33, 34</sup>, and

fish<sup>35-37</sup>. Mycobacteria have also been isolated from meat<sup>38</sup> and from raw<sup>24, 39, 40</sup> and pasteurized<sup>41</sup> milk. Mycobacterial contamination of milk seems to come directly from environmental sources (presumably from soil or water) and not from the cow<sup>41</sup>.

Early studies<sup>20, 21</sup> of soil mycobacteria found notable differences in the mycobacterial flora of soil samples from two states of the U.S., Ohio and Georgia, with *M. fortuitum* being more common than *M. avium* in Ohio and vice versa in Georgia. They also demonstrated that mycobacteria were common in muds and loams but could not be isolated from clay or sand.

The most systematic study<sup>42</sup> to date of the ecology of environmental mycobacteria was carried out in Uganda. The work was initiated in an attempt to determine the source of buruli disease, an ulcerating skin disease caused by *M. ulcerans* and common in certain parts of Uganda. Initially, samples of a grass (*Echinochloa pyramidalis*) which appeared to have a distribution similar to that of buruli disease<sup>43</sup>, were collected from dry grassland and from both permanent and seasonal swamps in several regions

<sup>16</sup> Davis, J. B., et al. *M. paraffinicum* n. sp., a bacterium isolated from soil. Appl. Microbiol. **4** (1956) 310-315.

<sup>17</sup> Kazda, J. Occurrence of non-cultivable acid-fast bacilli in the environment and their relationship to *M. leprae*. Lepr. Rev. **52** Suppl. (1981) 85-91.

<sup>18</sup> Tsukamura, M. A group of mycobacteria from soil sources resembling nonphotochromogens (group III). A description of *M. nonchromogenicum*. Med. Biol. (Tokyo) **71** (1965) 110-113.

<sup>19</sup> Tsukamura, M. Two types of slowly growing, non-photochromogenic mycobacteria obtained from soil by the mouse passage method: *M. terrae* and *M. novum*. Jpn. J. Microbiol. **11** (1967) 163-172.

<sup>20</sup> Wolinsky, E. and Rynearson, T. Mycobacterial flora of soil. Am. Rev. Respir. Dis. **94** (1966) 478-484.

<sup>21</sup> Wolinsky, E. and Rynearson, T. Mycobacteria in soil and their relation to disease-associated strains. Am. Rev. Respir. Dis. **97** (1968) 1032-1037.

<sup>22</sup> Wayne, L. G. Classification and identification of mycobacteria. III. Species within group III. Am. Rev. Respir. Dis. **93** (1966) 919-928.

<sup>23</sup> Richmond, L. and Cummings, M. M. An evaluation of methods of testing the virulence of acid-fast bacilli. Am. Rev. Tuberc. **62** (1950) 632-637.

<sup>24</sup> Kubica, G. P., et al. A method for the isolation of unclassified acid-fast bacilli from soil and water. Am. Rev. Respir. Dis. **88** (1963) 718-720.

<sup>25</sup> Graybill, J. R., et al. Disseminated mycobacteriosis due to *M. abscessus* in two recipients of renal homografts. Am. Rev. Respir. Dis. **109** (1974) 4-10.

<sup>26</sup> Bailey, R. K., et al. The isolation of high-catalase *Mycobacterium kansasii* from tap water. Am. Rev. Respir. Dis. **101** (1970) 430-431.

<sup>27</sup> Bullin, C. H., et al. Isolation of *M. xenopi* from water taps. J. Hyg. **68** (1970) 97-100.

<sup>28</sup> Wayne, L. G., et al. Classification and identification of mycobacteria. IV. Some important scotochromogens. Am. Rev. Respir. Dis. **96** (1967) 88-95.

<sup>29</sup> Zeligman, I. *Mycobacterium marinum* granuloma. A disease acquired in the tributaries of Chesapeake Bay. Arch. Dermatol. **106** (1972) 26-31.

<sup>30</sup> Marks, J. Aspects of the epidemiology of infection by the "anonymous" mycobacteria. Proc. R. Soc. Med. **57** (1964) 479-480.

<sup>31</sup> Strauss, I. and Gamaleia, N. Recherches experimentales sur la tuberculose; la tuberculose humaine, sa distinction de la tuberculose des oiseaux. Arch. Med. Exp. Anat. Pathol. **3** (1891) 457-484.

<sup>32</sup> Aronson, J. D., et al. A twenty-year appraisal of BCG vaccination in the control of tuberculosis. Arch. Intern. Med. **101** (1958) 881-893.

<sup>33</sup> Küster, E. Über Kaltblütertuberculose. Münch. Med. Wochenschr. **52** (1905) 57-59.

<sup>34</sup> Schwabacher, H. A strain of *Mycobacterium* isolated from skin lesions of a cold-blood animal, *Xenopus laevis*, and its relation to atypical acid-fast bacilli occurring in man. J. Hyg. **57** (1959) 57-67.

<sup>35</sup> Aronson, J. D. Spontaneous tuberculosis in salt water fish. J. Infect. Dis. **39** (1926) 315-320.

<sup>36</sup> Baker, J. A. and Hagan, W. A. Tuberculosis of the Mexican platyfish (*Platypoecilus maculatus*). J. Infect. Dis. **70** (1942) 248-252.

<sup>37</sup> Ross, J. A. *M. salmoniphilum* sp. nov. from salmonoid fishes. Am. Rev. Respir. Dis. **81** (1960) 241-250.

<sup>38</sup> Tison, F., et al. Techniques and results of a search for mycobacteria in meat. Ann. Inst. Pasteur Lille **17** (1966) 155-160.

<sup>39</sup> Chapman, J. S., et al. Isolation of mycobacteria from raw milk. Am. Rev. Respir. Dis. **91** (1963) 351-355.

<sup>40</sup> Tacquet, A., et al. Techniques for the detection of mycobacteria in milk and dairy produce. Ann. Inst. Pasteur Lille **17** (1966) 161-171.

<sup>41</sup> Chapman, J. S., et al. Isolation of atypical mycobacteria from pasteurized milk. Am. Rev. Respir. Dis. **98** (1968) 1052-1054.

<sup>42</sup> Stanford, J. L. and Paul, R. C. A preliminary report on some studies of environmental mycobacteria from Uganda. Ann. Soc. Belg. Med. Trop. **53** (1973) 389-393.

<sup>43</sup> Barker, D. J. P. Epidemiology of *Mycobacterium ulcerans* infection. Trans. R. Soc. Trop. Med. Hyg. **61** (1973) 43-50.

of Uganda. The rate of isolation of mycobacteria from the samples was highest (41%) in those from permanent swampland, somewhat less in those from seasonal swamps (15%), and lowest in those from grassland (9%). Further study using samples of mud from the edges of permanent swamps led to the production of a map showing the geographical distribution of six species of environmental mycobacteria in Ugandan soils. *M. gordonae* and *M. nonchromogenicum* appeared to be the principal mycobacteria in the Ugandan environment, and *M. vaccae* was apparently limited in distribution to the areas where buruli ulcer disease is endemic. Although *M. ulcerans* itself was not found in the Ugandan environment, the researchers (Stanford and Paul<sup>42</sup>) make the interesting observation that *M. ulcerans* infection seems to be limited to regions in which swamplands are slightly acidic (pH 6.1–6.9).

Research on environmental mycobacteria which could perhaps have potentially devastating effects on currently orthodox views on the biology of *M. leprae* and on the mode of transmission of leprosy is at present being undertaken in the German Federal Republic by Kazda<sup>17, 44</sup>. He has isolated noncultivable acid-fast bacilli (NC AFB) from a variety of environmental sources (sphagnum moss, soil, and water) from presently or recently leprosy-endemic areas around the world. He claims that at least two strains so isolated are indistinguishable from *M. leprae* in their behavior in mouse foot pads and armadillos, although this does not prove that they are capable of causing leprosy in humans. He has also investigated the ecology of NC AFB living in association with sphagnum moss in Norway, and has shown that the largest number of NC AFB are found on the surface of the so-called "gray layer" of sphagnum vegetation. Kazda claims<sup>17</sup> to have produced a sterile culture medium from this gray layer which is capable of supporting growth in this usually noncultivable AFB.

**Epidemiology of human mycobacterial infections.** While some species of environ-

mental mycobacteria may be entirely free living or may simply colonize human body surfaces, most species can, under certain conditions, cause human infection. The crucial difference between infection and colonization has been taken by some<sup>45</sup> to be that the former induces some functional or structural change, e.g., immunological sensitization, which the latter does not. However, it must be noted that sensitization to mycobacterial antigens might occur without colonization—if mycobacteria are swallowed, perhaps. Mycobacterial infections may themselves be manifested in frank disease or may remain clinically quite inapparent. Disease caused by the environmental mycobacteria fall into four main categories: 1) pulmonary infection (often similar to pulmonary tuberculosis), 2) cutaneous infection (for example, buruli ulcer and swimming pool granuloma), 3) lymphadenitis (usually cervical), and 4) disseminated infection (Table 2). The literature on the subject of diseases due to environmental mycobacteria is vast, and an exhaustive list of references may be found in a review by Barksdale and Kim<sup>46</sup>.

For some mycobacterial diseases the source and portal of entry of infection are well characterized. For example, swimming pool granuloma is caused by the infection by *M. marinum* of abrasions sustained from the sides of swimming pools<sup>47, 48</sup> or tropical fish tanks<sup>49, 50</sup>. Similarly, abscesses may result from the introduction of *M. chelonae* into the skin by injections<sup>51, 52</sup> or other

<sup>45</sup> Wolinsky, E. When is infection disease? Rev. Infect. Dis. 3 (1981) 1025–1027.

<sup>46</sup> Barksdale, L. and Kim, K.-S. *Mycobacterium*. Bacteriol. Rev. 41 (1977) 217–372.

<sup>47</sup> Linell, F. and Norden, A. *M. balnei*, a new acid-fast bacillus occurring in swimming pools and capable of producing skin lesions in humans. Acta Tuberc. Scand. 33 Suppl. (1954) 1–85.

<sup>48</sup> Morgan, J. K. and Blowers, R. Swimming pool granuloma in Britain. Lancet 1 (1964) 1034–1036.

<sup>49</sup> Barrow, G. I. and Hewitt, M. Skin infection with *M. marinum* from a tropical skin tank. Br. Med. J. 2 (1971) 505–506.

<sup>50</sup> Swift, S. and Cohen, H. Granulomas of the skin due to *M. balnei* after abrasions from a fish tank. N. Engl. J. Med. 267 (1962) 1244–1246.

<sup>51</sup> Borghans, J. G. A. and Stanford, J. L. *M. chelonae* in abscesses after injection of diphtheria-pertussis-tetanus-polio vaccine. Am. Rev. Respir. Dis. 107 (1973) 1–8.

<sup>44</sup> Kazda, J., Irgens, L. M. and Müller, K. Isolation of non-cultivable acid-fast bacilli in sphagnum and moss vegetation by foot pad technique in mice. Int. J. Lepr. 48 (1980) 1–6.

TABLE 2. Major categories of human disease caused by selected species of environmental mycobacteria.<sup>a</sup>

Mycobacteria	Lung disease	Skin disease	Lymphadenitis	Disseminated	Other
Slow growers	<i>M. avium</i> <sup>b</sup>	<i>M. marinum</i>	<i>M. avium</i> <sup>b</sup>	<i>M. avium</i> <sup>b</sup>	<i>M. avium</i>
	<i>M. kansasii</i>	<i>M. ulcerans</i>	<i>M. scrofulaceum</i>	<i>M. kansasii</i>	<i>M. kansasii</i>
	<i>M. scrofulaceum</i>			<i>M. nonchromogenicum</i>	<i>M. marinum</i>
	<i>M. xenopi</i>				
Fast growers	<i>M. chelonae</i>	<i>M. chelonae</i>	<i>M. chelonae</i>	<i>M. chelonae</i>	<i>M. fortuitum</i>
	<i>M. fortuitum</i>	<i>M. fortuitum</i>		<i>M. fortuitum</i>	

<sup>a</sup> Based on Table 4, Barksdale and Kim<sup>46</sup>.

<sup>b</sup> Includes *M. intracellulare*.

penetrating<sup>53</sup> wounds. After a detailed study<sup>43</sup> of the epidemiology of *M. ulcerans* infection in Uganda, Barker suggested that buruli ulcer disease is caused by the introduction of *M. ulcerans* into the skin via abrasions caused by marshland grasses. The portal of entry for mycobacteria causing cervical lymphadenitis is unknown, although some<sup>3</sup> have suggested the ingestion of soil, particularly by infants (geophagia), may be involved. It would seem likely that pulmonary mycobacterial disease is caused by inhalation of environmental mycobacteria. Geographical variations occur in the prevalence of environmental mycobacterial disease. For example, *M. xenopi* infection although relatively common in Europe is almost unknown in the U.S.A.<sup>54</sup>, and within Britain the disease may be found in the south and west of England but is unknown in the midlands or the north<sup>55</sup>. Ecological factors are probably involved here.

Infection by environmental mycobacteria is often clinically inapparent, and such subclinical infection may be detected by skin tests using purified protein derivative (PPD) prepared from each particular species. An early study<sup>56</sup> of skin test reactivity to a

reagent derived from *M. avium* carried out on U.S. Navy recruits showed a high prevalence of sensitivity among those from the southern United States in general and a particularly high prevalence (65%) among those from the southeastern states. Since reported cases of *M. avium* infection were nowhere near as common as this, one must conclude that subclinical infection is the most common outcome of contact with *M. avium* in this region.

The results of trials<sup>8</sup> in Uganda, using multiple skin testing with a range of mycobacterial antigens, showed that subclinical infection by environmental mycobacteria sufficient to sensitize individuals to homologous PPD must occur frequently in that region, because for each and every one of the 12 species of environmental mycobacteria under investigation, there were at least some individuals who showed prior sensitization to that species. Response rates varied both according to species (between the lowest rate, 10%, for *M. ulcerans* and the highest rates for *M. chelonae* and for a species isolated from the East African environment, temporarily designated *M. A.*, both 70%) and according to the region (the highest overall rate in the Kyoga area and the lowest in Toro)<sup>8</sup>. The results of these skin test studies document the prevalence of past or present infection, predominantly subclinical, by environmental mycobacteria. Stanford and his colleagues<sup>8</sup> note that these results fit in well with what is already known about the distribution of environmental mycobacteria and nontuberculous mycobacterial disease in Uganda. They suggest that indirect surveys of environmental mycobacteria in a given region may be performed by multiple skin testing of the pop-

<sup>52</sup> Inman, P. M., et al. Outbreak of injection abscesses due to *M. abscessus*. Arch. Dermatol. **100** (1969) 141-147.

<sup>53</sup> Gangadharam, P. R. and Hsu, K. H. K. *M. abscessus* infection in a puncture wound. Am. Rev. Respir. Dis. **106** (1972) 275-277.

<sup>54</sup> Elston, H. R. and Duffy, J. P. *M. xenopi* and mycobacteriosis. Am. Rev. Respir. Dis. **108** (1973) 944-949.

<sup>55</sup> Marks, J. and Schwabacher, H. Infection due to *M. xenopi*. Br. Med. J. **1** (1965) 32-33.

<sup>56</sup> Edwards, P. Q. and Edwards, L. B. The story of the tuberculin test from an epidemiological viewpoint. Am. Rev. Respir. Dis. **81** Suppl. (1960) 33.

ulation, and that such surveys may be cheaper and quicker than direct bacteriological studies.

Further studies<sup>57</sup> along lines similar to the first Ugandan study were carried out in three regions of the world—East Africa, Burma, and Libya—using some 25 skin test reagents derived from environmental mycobacteria. The results showed that even among those individuals who showed no response to antigens in the skin test reagent derived from *M. tuberculosis*, there was a substantial proportion of responders to one or more other mycobacterial reagents. In other words, infection by environmental species had occurred commonly in all three regions.

The fact that subclinical infection by one or more species of environmental mycobacteria is common in the tropics has been confirmed by other studies<sup>58</sup>. A study in Haiti<sup>59</sup> showed that over half the population tested had been infected by at least one of the five species investigated, and evidence of infection by more than one species of mycobacteria was found in over a quarter of the subjects tested. Low-grade tuberculin sensitivity indicative of previous or present infection by environmental mycobacteria is extremely common in most of the Indian population<sup>5</sup> except for those individuals living in a few areas at high altitudes. The Chingleput trial<sup>5</sup> (see below) showed that in the area tested over 95% of the population has become sensitized to PPD-B (a skin test reagent derived from a strain belonging to the *M. avium-intracellulare-scrofulaceum* complex) by the time they had reached adulthood.

Thus, it is now well established from epidemiological and bacteriological surveys that contact between humans and environmental mycobacteria is common in many tropical and subtropical regions. The question remains as to what relevance this has to leprosy and tuberculosis control.

<sup>57</sup> Shield, M. J., *et al.* Multiple skin testing of tuberculosis patients with a range of new tuberculins, and a comparison with leprosy and *M. ulcerans* infection. *J. Hyg. (Camb.)* **78** (1977) 331–348.

<sup>58</sup> Nyunt, T., *et al.* Sensitivity to tuberculin and to a non-mammalian sensiti in Mandalay District, Burma. *Bull. WHO* **52** (1975) 63–67.

<sup>59</sup> Vandiviere, H. M., *et al.* Profiles of skin test reactivity to antigens of various mycobacterial species in

### Efficacy of BCG vaccination in humans—variable results and a variety of explanatory hypotheses

**Results of early trials.** While the protective effect of BCG vaccination against tuberculosis or leprosy may be shown using animal models of human disease, controlled field trials of the efficacy of BCG vaccination against both diseases are of much greater clinical relevance. The results of such trials have been remarkably variable<sup>4</sup> (Table 3) and, even before the recent Indian trial, there was a dishearteningly low level of protection shown by several trials. Three trials<sup>60–62</sup> of the efficacy of BCG vaccination undertaken in the United States all showed little or no protection against tuberculosis and, similarly, a trial in Burma<sup>63</sup> showed little or no effect of BCG vaccination in protecting against leprosy in the population tested. Two other trials, one in Puerto Rico<sup>64</sup> and the other in India<sup>65, 66</sup>, showed only a moderate protective effect of BCG vaccination against tuberculosis.

Set against these discouraging results, three trials of BCG vaccination against tuberculosis—two in the U.S.<sup>32, 67</sup> and one in the United Kingdom<sup>68–70</sup>—showed protec-

a human population and in experimental infections. *Tubercle* **61** (1980) 245–257.

<sup>60</sup> Bettag, O. L., *et al.* BCG study at a state school for mentally retarded. *Dis. Chest* **45** (1964) 503–507.

<sup>61</sup> Comstock, G. W. and Palmer, C. E. Long-term results of BCG vaccination in the southern United States. *Am. Rev. Respir. Dis.* **93** (1966) 171–183.

<sup>62</sup> Comstock, G. W. and Webster, R. G. Tuberculosis studies in Muscogee County, Georgia. *Am. Rev. Respir. Dis.* **100** (1969) 839–845.

<sup>63</sup> Bechelli, L. M., *et al.* BCG vaccination of children against leprosy: 9-year findings of the controlled WHO trial in Burma. *Bull. WHO* **51** (1974) 93–99.

<sup>64</sup> Palmer, C. E., *et al.* Community trials of BCG vaccination. *Am. Rev. Tuberc. Pulm. Dis.* **77** (1958) 877–891.

<sup>65</sup> Frimodt Møller, J., *et al.* Observations on the protective effect of BCG vaccination in a South Indian rural population. *Bull. WHO* **30** (1964) 545–574.

<sup>66</sup> Frimodt Møller, J., *et al.* Observations on the protective effect of BCG vaccination in a South Indian rural population: fourth report. *Bull. Int. Union Tuberc.* **48** (1973) 40–52.

<sup>67</sup> Rosenthal, S. R., *et al.* BCG vaccination against tuberculosis in Chicago. A twenty-year study statistically analyzed. *Pediatrics* **28** (1961) 622–641.

<sup>68</sup> Medical Research Council. BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life; first report. *Br. Med. J.* **1** (1956) 413–427.

TABLE 3. Results of controlled trials of the efficacy of BCG vaccination in humans.<sup>a</sup>

Population group	Period of intake	Disease under consideration	Overall protective efficacy <sup>b</sup>
North American Indians Chicago, U.S.A.	1935–1938	Tuberculosis	80%
Infants	1937–1948	Tuberculosis	75%
Georgia, U.S.A. School children	1947	Tuberculosis	0%
Illinois, U.S.A. School children	1947–1948	Tuberculosis	0%
Puerto Rico General population	1949–1951	Tuberculosis	31%
Georgia and Alabama, U.S.A. General population	1950–1952	Tuberculosis	14% <sup>c</sup>
United Kingdom Urban school leavers	1950–1952	Tuberculosis	78%
South India Rural population	1950–1955	Tuberculosis	31%
Uganda Children	1960–1962	Leprosy	87%
South India Rural population	1968–1971	Tuberculosis	0%
Burma Children	1964–1973	Leprosy	17% <sup>c</sup>

<sup>a</sup> Based on Table 1 in ten Dam, *et al.*<sup>74</sup> and other sources<sup>4,5,63,72</sup>.

<sup>b</sup> Defined as  $u - v/u$ , where  $u$  = prevalence of disease in the unvaccinated population and  $v$  = prevalence of disease in the vaccinated population.

<sup>c</sup> Not statistically significant.

tion rates of around 80%. A trial<sup>71–73</sup> of BCG vaccine against leprosy in Uganda achieved a protection rate of some 87%.

**Attempts to explain the early results.** Many potential sources of disparity were considered<sup>74</sup>, and many were excluded, as explanations of these conflicting results. Methodological shortcomings were excluded,

as were genotypical or phenotypical differences between the populations tested<sup>74</sup>. Nutritional differences were ruled out by the high efficacy of vaccination found among North American Indians<sup>32</sup> and by the lack of association of efficacy with thickness of subcutaneous fat in the Georgia-Alabama trial<sup>61</sup>. In fact, before the latest results from the Chingleput trial were known attention was focussed on two possible explanations for the variability of protection afforded by BCG vaccination: a) either there were differences in the protective efficacy of the various vaccines used in the different trials, or b) variability in the prevalence of previous sensitization to environmental mycobacteria in the areas which were compared obscured the effects of BCG vaccination. The former argument can be subdivided into two propositions: a) that either significant variations in the preparation, handling, and application of vaccines occurred; or b) that variability might occur in the actual protective efficacy of the strain of BCG used.

That faulty preparation of the vaccine

<sup>69</sup> Medical Research Council. BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life; second report. *Br. Med. J.* **2** (1959) 379–396.

<sup>70</sup> Medical Research Council. BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life; third report. *Br. Med. J.* **1** (1963) 973–978.

<sup>71</sup> Brown, J. A. K. and Stone, M. M. BCG vaccination against leprosy: first results of a trial in Uganda. *Br. Med. J.* **1** (1966) 7–14.

<sup>72</sup> Brown, J. A. K., *et al.* BCG vaccination of children against leprosy in Uganda: results at end of second follow-up. *Br. Med. J.* **1** (1968) 24–27.

<sup>73</sup> Brown, J. A. K., *et al.* Trial of BCG vaccination against leprosy in Uganda. *Lepr. Rev.* **40** (1969) 3–7.

<sup>74</sup> ten Dam, H. G., *et al.* Present knowledge of immunization against tuberculosis. *Bull. WHO* **54** (1976) 255–269.

might have a pronounced effect was shown in the North American Indian trial<sup>74</sup> in which a poor culture medium was used for some time. An appreciable difference in both conversion rate and protective efficacy was noted between those vaccinated with the poorly cultured vaccine and those who had received the adequately cultured BCG. Differences in the mode of administration of vaccine were thought by some to be at fault. It was supposed that multiple puncture application failed to administer an effective dose. On the other hand, this does not explain the partial failure of the Puerto Rico trial in which an alternative method was used.

That variability in the protection offered by various strains of BCG might occur has been confirmed in animal models<sup>75-77</sup> and the potency differences found correlate well with the observed differences in immunogenicity and virulence of various strains in animal experiments<sup>77</sup>. Furthermore, these potency differences were closely associated with the observed differences in the efficacy of vaccination in three of the controlled trials in humans<sup>74</sup>. It was also noted that in the three U.S. trials, where no protection against tuberculosis was found, the vaccines used were prepared by the same laboratory<sup>5</sup>. On the other hand, this laboratory produced a number of different strains at different times<sup>74</sup>. It is not possible to say whether the strains used in these three trials were the same. A fourth trial, the Chicago trial, also used a vaccine prepared by this same laboratory but the results showed a high protective efficacy of BCG vaccination. In any case, until recently the basic proposition that variability in the efficacy of BCG vaccination was due to variability in the efficacy of the vaccine, or in its mode of administration, seemed to many researchers to be the most likely explanation of the disparities

found. All that changed when the results of the most recent trial were made public.

**Chingleput, South India trial<sup>5</sup>.** The trial took place in South India and was at first under the administration of the National Tuberculosis Institute, Bangalore, and later under the Indian Council of Medical Research. The objectives of the trial were to obtain a precise estimate of the protective effect of BCG vaccination in the noninfected and in those already infected at intake, and to determine the effects of the BCG strain and the dosage of vaccine administered on protective efficacy, as well as providing epidemiological data on tuberculosis in the community. It had originally been hoped to also look for a difference in the effect of BCG vaccination in one population where exposure to environmental mycobacteria was common and in one where it was rare, but no suitable area with a low prevalence of nonspecific tuberculin sensitivity could be found in India. The area chosen for the trial was an area within Chingleput District, near Madras in Tamil Nadu State, Southern India. The study area had a population of around 360,000, with the entire resident population over the age of one month eligible for inclusion in the trial. The trial was controlled and double blind, with each person over one year old tested with 3 IU of tuberculin PPD-S and 10 "units" of tuberculin PPD-B (derived from *M. intracellulare*) at the outset of the trial. Each subsequently either received one of two strains of BCG vaccine (prepared from two seed lots—the "Paris strain" 1173 P2 and the "Copenhagen strain" 1131) at one of two strengths (the usual 0.1 mg in 0.1 ml or the weaker 0.001 mg in 0.1 ml), or a placebo injection—all of these variables were allotted randomly. All individuals aged ten years or older were given a chest X-ray. For those whose X-ray report was abnormal, two sputum samples were collected and examined bacteriologically. The intake ran from July 1968 until March 1971.

In July 1980 the results of the first 7½ years of followup of the trial were published<sup>5</sup> in full for the first time. To the surprise and dismay of all concerned, the distribution of bacillary pulmonary tuberculosis among the initially tuberculin-negative population showed that BCG vaccination, irrespective

<sup>75</sup> Gheorghiu, M. and Lagrange, P. H. Viability, heat stability and immunogenicity of four BCG vaccines prepared from four BCG strains. *Ann. Immunol. (Paris)* **134C** (1983) 125-147.

<sup>76</sup> Mackaness, G. B., *et al.* Immunopotentiality with BCG. I. Immune response to different strains and preparations. *J. Natl. Cancer Inst.* **51** (1973) 1655-1667.

<sup>77</sup> Willis, H. S. and Vandiviere, M. R. The heterogeneity of BCG. *Am. Rev. Respir. Dis.* **84** (1961) 288-290.

of the strain or dosage used, had given no protection at all to this group of people during the period under consideration. Furthermore, neither were those who were tuberculin positive at the outset of the trial protected by vaccination. Other interesting findings included: a) incidence of disease was much lower among those not infected at the outset as compared to those infected, b) incidence of disease was nearly three times more common in males, and c) the initially strong delayed hypersensitivity to PPD-S due to BCG vaccination in the tuberculin-negative group was seen to decrease between re-testings at 2½ months and 2½ years, although no further decline was noted thereafter and the average level of sensitivity in the vaccinated group remained considerably higher than in the placebo group.

**Some current hypotheses.** The former orthodox explanation of the partial or complete failure of BCG vaccination to protect against tuberculosis in certain trials, namely, that the vaccines or their mode of administration had been faulty, was almost completely discredited by the results of the Chingleput trial. Numerous technological advances<sup>78</sup> had been made since the earlier trials in an attempt to guarantee the quality of the vaccines and the mode of vaccination. Freeze-dried vaccine was introduced because it keeps better and eliminates changes due to mutation and selection. Strict quality control of vaccines was undertaken, and two strains showing consistently high activity in animals and good immunogenicity in man were selected for trial<sup>5</sup>. One of the two strains, the "Copenhagen strain," had been used earlier in the British trial<sup>68-70</sup> where, as stated above, a high degree of protection was demonstrated. Furthermore, a WHO Scientific Working Group could find no defect in the design of the trial or in its execution<sup>79</sup>. Thus, considering all the detailed work that went into the planning and execution of this trial, it seems highly unlikely that the researchers involved should

have produced, as ten Dam and Pio<sup>78</sup> put it, "the worst vaccines ever."

Given this fact, the search for an alternative explanation of the results was initiated. A Scientific Group on Vaccination Against Tuberculosis, jointly sponsored by the Indian Council of Medical Research and WHO, met to discuss the matter in the spring of 1980. In their report<sup>79</sup> published in 1981, several hypotheses were put forward as explanations for the negative result of the Chingleput trial, with suggestions for future research. Since then, others<sup>6, 78</sup> have also reviewed the results of the trial and have added to or expanded upon the ideas of the Scientific Group. A number of these hypotheses are reviewed below; consideration of certain other hypotheses, namely those concerning the environmental mycobacteria, are discussed later in this review.

Firstly, although the reasons for believing that faulty vaccines were not to blame have been outlined above, it is still worth noting that the Chingleput trial was the first trial of its kind in which freeze-dried vaccine was used<sup>6</sup>. However, given the expert authority<sup>80</sup> that freeze-dried vaccines "are to be preferred under most all circumstances," it seems unlikely that they are the source of the problem.

A second hypothesis<sup>79</sup> suggests that the BCG vaccines used in the Chingleput trial were not able to protect against the South Indian variant of *M. tuberculosis* which encompasses a number of strains encountered in the city of Madras that have a low virulence in the guinea pig. Another hypothesis<sup>79</sup> based on the high prevalence rate of this variant in the region consists of three tentative propositions: a) that the less virulent variant might nonetheless be highly infectious, infecting a large proportion of the placebo group; b) that infection by this variant might protect individuals against subsequent superinfection by highly virulent strains of *M. tuberculosis*; and c) that BCG vaccination might interfere with this phenomenon, perhaps by protecting against infection by the less virulent strains but not against infection with highly virulent *M. tuberculosis*.

<sup>78</sup> ten Dam, H. G. and Pio, A. Pathogenesis of tuberculosis and effectiveness of BCG vaccination. *Tubercle* 63 (1982) 225-233.

<sup>79</sup> Vaccination against tuberculosis. Report of an ICMR/WHO Scientific Group. WHO Tech. Rep. Ser. No. 651, 1980.

<sup>80</sup> WHO Expert Committee on Tuberculosis. Ninth Report. WHO Tech. Rep. Ser. No. 552, 1974.

Taken together, these propositions might add up to produce no significant differences between the control and vaccinated groups. Of these two hypotheses, the former would seem rather implausible. BCG vaccination has been shown to afford protection against distantly related mycobacterial species such as *M. leprae*<sup>73</sup> and even, in animal models, against *Listeria monocytogenes*<sup>81</sup>, and so one would expect it to protect humans against all strains of *M. tuberculosis*, if it is effective at all. The assumptions of the latter, more complicated hypothesis are as yet entirely speculative and unsubstantiated.

Another possible explanation is that the tuberculosis diagnosed in the South Indian trial is predominantly of the exogenous-superinfection type<sup>78, 79</sup>, and thus those previously vaccinated could not be expected to have any advantage over those not vaccinated when exogenous reinfection occurred. Much evidence for this hypothesis, drawn from analysis of both the results of the Indian trial and the results of earlier trials, has been collected in a tightly argued paper by ten Dam and Pio<sup>78</sup>. They suggest that protection by BCG vaccination will only occur in those environments where, unlike in India, primary or evolutive disease is common, which may be gauged from the proportions of extrapulmonary disease found (33% in England, 10% in Puerto Rico, and 0% in India). This striking hypothesis certainly provides a fairly convincing explanation of the facts and merits further investigation.

Yet another hypothesis<sup>79</sup> is that there may have been some genetically or environmentally determined suppression of the immune response in the population considered. That genetic factors may be important would seem unlikely, especially since variations in the efficacy of BCG vaccination have occurred in ethnically similar populations, i.e., in the U.S. Environmental factors cannot be excluded, but for a single environmental agent to act on such a large population would be unlikely unless, of course, it were some kind of infectious agent.

### Possible effects of environmental mycobacteria on the efficacy of BCG vaccination

**An early hypothesis.** Soon after it had become obvious in the 1960s that there were variations in the efficacy of BCG vaccination, as shown by the U.K., Puerto Rico, and Georgia-Alabama trials, the hypothesis<sup>82</sup> was advanced that the differences could be accounted for by differences in the prevalence of sensitization to environmental mycobacteria in the areas compared. More specifically, it was proposed that in the situations where BCG vaccination apparently failed, what had actually happened was that both the vaccinated and unvaccinated populations were already maximally immunized against tuberculosis by contact with environmental mycobacteria and BCG could add no more protection to that already present. As has been noted above, the prevalence of sensitization to environmental mycobacteria is extremely high in many tropical areas and thus, at first sight, this hypothesis seems quite plausible. Furthermore, experiments<sup>82</sup> using guinea pigs showed that certain species of environmental mycobacteria could indeed immunize against tuberculosis, with varying degrees of effectiveness as compared with BCG (*M. kansasii* showed 70% of the protection afforded by BCG, *M. avium* 50% of the protection, and *M. fortuitum* 15%).

Protection was apparently a quantitative phenomenon but with a maximum such that if BCG vaccination were superimposed on the protection afforded by sensitization to an environmental mycobacterial species, the overall protection would be no more than if BCG were used alone. In a controlled trial, BCG vaccination would apparently only show a protective effect equal to the difference between the protection already present and the maximum possible protection.

This hypothesis was initially challenged by other researchers<sup>8</sup> who pointed out that in animals the protective effects of sensitization to environmental mycobacteria

<sup>81</sup> Bennedsen, J. and Olesen Larsen, S. The BCG-induced resistance to Listeriosis. Acta Pathol. Microbiol. Scand. **83** (1975) 377-382.

<sup>82</sup> Palmer, C. E. and Long, M. W. Effects of infection with atypical mycobacteria on BCG vaccination and tuberculosis. Am. Rev. Respir. Dis. **94** (1966) 553-568.

TABLE 4. Differentiation between two types of responses to mycobacterial antigens in humans and experimental animals.<sup>a</sup>

Type of response	Differentiating properties in		
	Mice	Guinea pigs	Humans
Listeria	Peak inducibility at 10–12 days Skin test response peaks at 18–23 hr	Non-necrotizing Inducible after 10–20 days	Early (12–18 hr) onset Pink, itchy, soft-edged induration
Koch	Peak inducibility at 4–6 weeks Skin test response peaks at 40–48 hr	Necrotizing Inducible after 30+ days	Late (max. at 72–96 hr) onset Purple/red, tender, hard-edged induration

<sup>a</sup> Based on data from Rook and Stanford<sup>85,105</sup> and Stanford<sup>9</sup>.

were simply too small, compared to BCG, to account for the results in humans. A protective effect of around 95% of that of BCG would be needed to explain the results of the Georgia–Alabama trial, according to the calculations. Thus, this hypothesis was dismissed until recently because, although it sounded plausible in theory, it failed to tally with the observed facts.

More recently, this hypothesis has been restored to credibility by work with guinea pigs. These experiments<sup>83</sup> showed that vaccination with *M. avium/intracellulare* was able to protect against some (low virulence) strains of *M. tuberculosis* with an efficacy equal to that of BCG. Work in mice has shown similar results<sup>84</sup>. Thus, this hypothesis, albeit simple, must now rank as one of the most plausible explanations of the variable results of BCG vaccination in humans.

**A more sophisticated hypothesis: The Stanford/Rook hypothesis.** A more complex hypothesis to explain the variable efficacy of BCG vaccination has been formulated in recent years by Stanford and Rook of the Middlesex Hospital Medical School, London, England, and their colleagues. Experimental work in mice and a critical reappraisal of previous research led them to the belief that all earlier thinking about the delayed hypersensitivity phenom-

enon, as elicited by mycobacterial antigens in both humans and animals, had been confused because the phenomenon was a heterogeneous response, and earlier workers had failed to recognize this. Their experimental work led them to delineate two types of responses to mycobacteria in the sensitized mouse which were separable according to a large number of criteria<sup>9,85</sup>. Correlations were established with earlier work in the guinea pig<sup>85</sup> and with responses to skin test antigens in humans<sup>9</sup> (Table 4). One type of response, which Stanford and Rook identified with the form of skin test reactivity first described by Koch<sup>86</sup> in tuberculosis in guinea pigs, was termed the “Koch-type response”; the other type of response was called the “Listeria-type response,” since it had first been described by Mackaness<sup>87</sup> as occurring in immunized mice in response to a challenge by *Listeria monocytogenes*. The Koch-type response was found to be protective in mice against subcutaneous challenge by mycobacteria belonging to the species *M. avium* and *M. lepraemurium*; whereas it increased vulnerability to intravenous challenge by the same organisms.

<sup>85</sup> Rook, G. A. W. and Stanford, J. L. The relevance to protection of three forms of delayed skin-test response evoked by *M. leprae* and other mycobacteria in mice. Correlation with the classical work in the guinea pig. *Parasite Immunol.* **1** (1979) 111–123.

<sup>86</sup> Koch, R. Fortsetzung der Mittheilungen über ein Heilmittel gegen Tuberculose. *Dtsch. Med. Wochenschr.* **17** (1891) 101–102.

<sup>87</sup> Mackaness, G. B. The immunology of anti-tuberculous immunity. *Am. Rev. Respir. Dis.* **97** (1968) 337–344.

<sup>83</sup> Edwards, M. L., *et al.* Infection with *M. avium-intracellulare* and the protective effects of bacille Calmette-Guerin. *J. Infect. Dis.* **145** (1982) 733–741.

<sup>84</sup> Collins, F. M. Immunogenicity of various mycobacteria and the corresponding levels of cross-protection developed between species. *Infect. Immun.* **4** (1971) 688–696.

On the other hand, the Listeria-type response is able to protect against intravenous challenge with *M. avium* but is unable to prevent dissemination of *M. avium* and *M. lepraemurium* from subcutaneous sites<sup>7</sup>.

As noted in Table 4, Rook and Stanford claim to be able to identify two separate responses to skin test antigens in humans corresponding to the Koch- and Listeria-type responses. Analysis of skin test data shows that the type of response which is triggered varies according to the species of mycobacterium and the size of the challenge. Some species, e.g., *M. leprae*, *M. vaccae*, and *M. nonchromogenicum*, can only induce the Listeria-type response to homologous soluble skin test reagents. Others, such as *M. kansasii*, *M. scrofulaceum*, and *M. tuberculosis*, are capable of inducing either type of response according to their prevalence in the environment and the particular mix of species present<sup>88</sup>.

Rook and Stanford put forward their own hypothesis<sup>88</sup> in an attempt to explain the results of BCG vaccination trials. They reasoned that the first type of response developed upon encountering environmental mycobacteria is likely to be the Listeria type. They assumed that, provided there is no exposure to mycobacteria capable of inducing the Koch-type response, the individual is thus likely to be protected against subsequent disease. This protection is likely to be strengthened by BCG vaccination. On the other hand, if a Koch-type response has also been invoked in an individual, then that individual would be more susceptible to disease. This susceptibility would, if anything, be increased by BCG vaccination.

From this hypothesis, one would therefore expect the response to BCG vaccination to be highly variable between populations in different parts of the world, just as is found. Populations would vary in the degree of exposure to environmental mycobacteria and in the particular mix of species encountered.

In an attempt to explain the results of unsuccessful U.S. trials, Stanford, *et al.*<sup>88</sup> also invoke the earlier hypothesis that widespread exposure to environmental myco-

bacteria may afford maximal protection against disease and thus completely mask the effects of BCG vaccination. They interpret this to mean that widespread induction of strong Listeria-type reactions occurs in the areas under consideration. Stanford and Rook have refined their hypothesis to explain the poor efficacy of BCG vaccination in Burma and South India<sup>88, 89</sup>. They now point out that differences in the type of response evoked by one particular species, *M. scrofulaceum*, may underlie the differences in BCG efficacy. In Iran, where responses to the PPD "Scrofulin" are of the Listeria-type, Scrofulin positivity is associated with a high level of Leprosin-A positivity (Leprosin A is a PPD prepared from *M. leprae*) and this can be further increased by BCG vaccination. In contrast, in Burma, where responses to Scrofulin are often Koch-type, Scrofulin positivity is associated with a decrease in Leprosin A positivity among those vaccinated with BCG.

If Stanford and Rook are correct in their immunologically based hypothesis, then the pattern of distribution of environmental mycobacteria and the epidemiology of environmental mycobacterial infection throughout the world are important determinants of the efficacy of BCG vaccination in the control of leprosy and tuberculosis.

#### Environmental mycobacteria as a source of new vaccines

As stated above, certain species of environmental mycobacteria have been shown to be effective at immunizing guinea pigs against tuberculosis, although in the early experiments they were not found to be as potent as BCG<sup>82</sup>. On theoretical grounds, this is not unexpected. The original BCG strain was derived from a strain of *M. bovis* which is very closely related to *M. tuberculosis* and, in fact, is placed by some workers<sup>15</sup> within the same species. Thus, BCG would be expected to afford better protection against *M. tuberculosis* than would less closely related species. However, this *a priori* assumption has been challenged by more recent experiments<sup>83</sup> which showed that *M. avium* was able to protect guinea

<sup>88</sup> Stanford, J. L., *et al.* How environmental mycobacteria may predetermine the protective efficacy of BCG. *Tubercle* 62 (1981) 55-62.

<sup>89</sup> Stanford, J. L. A vaccine for leprosy. *Lepr. Rev.* 47 (1976) 87-91.

pigs against challenge with *M. tuberculosis* just as effectively as BCG. Thus, the question of whether a vaccine against *M. tuberculosis* of equal or greater efficacy than BCG could be prepared from environmental mycobacteria still remains.

On the other hand, even by *a priori* reasoning, environmental mycobacteria could be expected to provide a better vaccine than BCG against leprosy since *M. leprae* is not immunologically particularly closely related to *M. tuberculosis*. Our inability to grow *M. leprae in vitro* has meant that a vaccine against leprosy cannot be prepared from *M. leprae* in a way analogous to that by which BCG was derived from *M. bovis*<sup>90</sup>. Thus, interest continues in the possibility of deriving an effective antileprosy vaccine from the environmental mycobacteria. Attention has been focused on one species in particular—*M. vaccae*, a fast-growing species not known to be pathogenic in humans or animals. It seems to be immunologically particularly closely related to *M. leprae*. *M. leprae* and *M. vaccae* are so far unique in that they both lack group ii and group iii antigens<sup>9</sup>, and the “specific” immunological defect that lepomatous leprosy patients show towards *M. leprae* also applies, to a marked degree, towards *M. vaccae*<sup>91</sup>. Stanford has suggested that *M. vaccae* might provide an effective vaccine against leprosy<sup>89</sup>. On the other hand, out of a selection of mycobacteria investigated (including *M. vaccae*) only BCG provides an effective vaccine against challenges with *M. leprae* in the mouse foot pad model<sup>92</sup>.

#### Environmental mycobacteria as a source of interference in diagnosis and epidemiological investigation of leprosy and tuberculosis

Since the early days of tuberculin testing, it has been realized that in many populations there is some low-grade tuberculin sensitivity due to skin test crossreactivity between *M. tuberculosis* and the environ-

mental mycobacteria<sup>93, 94</sup>. More specific skin test reagents may soon be prepared using, for example, monoclonal antibody techniques to identify species-specific antigens. However, in the meantime, skin test cross-reactivity remains a considerable nuisance in evaluating the results of tuberculin testing.

Similarly, the whole range of newer immunological tests that have been introduced into the investigation of the epidemiology and immunology of leprosy and tuberculosis are limited by the requirement that they have to have very high sensitivities in order to be of any use in providing high predictive values for positive results, especially in areas of low endemicity<sup>95</sup>. For example, Bjune<sup>96</sup> notes that the applicability of the lymphocyte transformation test to epidemiological studies of subclinical leprosy is severely limited by the fact that the relevance of a positive response in a healthy individual is still not established, partly due to ignorance of the role of crossreactions due to contact with environmental mycobacteria. Uncertainties as to whether positive responses are due to crossreactions with environmental mycobacteria a) add to the doubts over the usefulness of new techniques, b) add to the complexities of evaluating immunological tests, and thus c) hold back research relevant to the control of leprosy and tuberculosis, particularly research relevant to early detection of disease.

#### DISCUSSION

In this essay a large amount of information has been surveyed, covering a number of distinct but related topics, yet one is still left with the unanswered question: What is the significance of the environmental mycobacteria for leprosy and tuberculosis con-

<sup>90</sup> Calmette, A. and Guerin, C. Sur quelques propriétés du bacille tuberculeux cultivé sur la bile. C. R. Seances Acad. Sci. **147** (1908) 1456–1459.

<sup>91</sup> Paul, R. C., *et al.* Multiple skin testing in leprosy. J. Hyg. (Camb.) **75** (1975) 57–68.

<sup>92</sup> Shepard, C. C., *et al.* Searches among mycobacterial cultures for antileprosy vaccines. Infect. Immun. **29** (1980) 1034–1039.

<sup>93</sup> Edwards, L. B., *et al.* Specific and nonspecific sensitivity in India. Indian J. Tuberc. **2** (1955) 66–71.

<sup>94</sup> Palmer, C. E., *et al.* Studies of pulmonary findings and antigen sensitivity among student nurses: geographic differences in sensitivity to tuberculin as evidence of nonspecific allergy. Public Health Rep. **65** (1950) 1111–1131.

<sup>95</sup> Kronvall, G. The potential of immunological tests as tools in the epidemiology of leprosy. Lepr. Rev. **52** Suppl. (1981) 207–219.

<sup>96</sup> Bjune, G. The lymphocyte transformation test in leprosy with special reference to its use in epidemiology. Lepr. Rev. **52** Suppl. (1981) 241–250.

trol? As stated earlier, two of the hypotheses put forward to explain the results of the Chingleput and other trials assign an important role to the environmental mycobacteria in interfering with the usefulness of BCG vaccination. If either hypothesis proves true, this could have important repercussions for the control of both leprosy and tuberculosis. In fact, both hypotheses could be true; they are not mutually exclusive. Contact with the environmental mycobacterial flora may be protective in one part of the world, yet increase susceptibility to disease in another region.

Both hypotheses are in agreement over the fact that the efficacy of BCG vaccination is related to the prevalence of contact with environmental mycobacteria—where there has been no such contact (for example, probably among urban populations in the industrialized world), BCG vaccination should prove effective. The two hypotheses disagree on the particular significance of contact with particular species. It is not yet known whether contact with environmental mycobacteria could be avoided in areas where they are common. If such contact could be avoided, or at least delayed, this could have important implications for predetermining the efficacy of BCG vaccination. The WHO Study Group<sup>97</sup> recommends that local factors which may influence the outcome of BCG vaccination measures should be taken into account. Yet we cannot predict whether BCG vaccination will prove effective in a given population with a high prevalence of sensitization to environmental mycobacteria, simply because we do not yet know whether either or both hypotheses are correct. In spite of this, current BCG vaccination policies, at least in the Third World, should be continued as the WHO Study Group recommends<sup>97</sup>. BCG vaccination, being one of the safest forms of vaccination, very seldom harms individuals. Given the limited health resources in the Third World, it may be the only weapon available for a community assault on tuberculosis and leprosy.

The Stanford/Rook hypothesis and the exogenous superinfection hypothesis also

share one important implication—that BCG vaccination will be most effective on infants and neonates. This is because either a) it will be able to act before exposure to certain environmental mycobacteria can induce the deleterious Koch-type response, or b) because it will be able to prevent the primary infection by the tubercle bacillus. Furthermore, a review<sup>98</sup> of the results of controlled trials and retrospective studies of BCG vaccination in the newborn suggests that the vaccine is highly effective under such circumstances. Thus, the material reviewed in this essay is in agreement with the suggestions of others<sup>6,98</sup> that the use of BCG vaccination in infants and neonates should be encouraged, both in the Third World and among high-risk groups in the West (for example, Asians<sup>99</sup> in Britain).

All three of the important hypotheses considered in this essay (the two hypotheses implicating environmental mycobacteria and the exogenous superinfection hypothesis) are likewise in agreement in implying that the efficacy of BCG vaccination may be high in the developed world yet low in the developing world. The very same factors, such as overcrowding, poor housing, and poor hygiene, which are known to be important in the spread of leprosy and tuberculosis may also decrease the efficacy of BCG vaccination. On the one hand, such factors may determine the degree of contact with and sensitization to the environmental mycobacteria. On the other hand, they might increase the risk of secondary infection tuberculosis. Thus, it may well be the case that to him “that hath shall be given, and from him that hath not, even that which he hath shall be taken away from him”<sup>100</sup>. In other words, BCG vaccination in adults may be effective only in those countries where there is a low prevalence rate of disease anyway, plus adequate health resources to control disease by other methods. It may prove ineffective in just those countries where, because of high prevalence rates and lack of health resources, it is most needed. Indeed,

<sup>97</sup> BCG vaccination policies. Report of a WHO Study Group. WHO Tech. Rep. Ser. No. 652, 1980.

<sup>98</sup> ten Dam, H. G. and Hitze, K. L. Does BCG vaccination protect the newborn and young infants. *Bull. WHO* 58 (1981) 37–44.

<sup>99</sup> BCG vaccination in the newborn. *Br. Med. J.* 2 (1980) 1445–1446.

<sup>100</sup> Luke 19:26.

in Britain there is discussion<sup>101</sup> as to whether universal BCG vaccination is still worthwhile, given the present falling prevalence rates of tuberculosis in the general population.

The future of BCG vaccination in the long-term control and eventual eradication of leprosy and tuberculosis remains questionable. Even where it is effective, other specific control measures, such as early case detection and treatment, chemoprophylaxis (already widely used in the U.S.<sup>102</sup>), and potentially the use of newer, better vaccines<sup>89, 103</sup>, may prove more important. In the long term, it may well be that "economic

development based on basic egalitarian principles is paramount for the final control of tuberculosis,"<sup>104</sup> and leprosy.

—Mark J. Pallen

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<sup>101</sup> BCG in Britain. *Br. Med. J.* **2** (1980) 825.

<sup>102</sup> Chemoprophylaxis for tuberculosis. *Tubercle* **61** (1981) 69–70.

<sup>103</sup> Convit, J., *et al.* Vaccination in leprosy—observations and interpretation. *Int. J. Lepr.* **48** (1980) 62–64.

<sup>104</sup> Tuberculosis control. Report of a joint IUAT/WHO Study Group. WHO Tech. Rep. Ser. No. 671, 1982.

<sup>105</sup> Rook, G. A. W. and Stanford, J. L. The heterogeneity of the immune response to mycobacteria and the relevance of the common antigens to their pathogenicity. *Ann. Immunol. (Paris)* **132D** (1981) 155–164.