The *In Vitro* Culture of the Leprosy Bacillus[1]

The leprosy bacillus (*Mycobacterium leprae*) was recognized in 1873[2], and was the first acid-fast bacterium to be described. Since then many attempts have been made to cultivate it *in vitro*. Although many successes have been claimed, growth has not been confirmed. More success has been achieved with animal models, where infection has been produced in mouse foot pads[3] and in the nine-banded armadillo[4]. Various hypotheses have been proposed to explain the repeated failures *in vitro*, including the idea that *M. leprae* is an obligate intracellular parasite, or that it has a dimorphic life cycle.

After a consideration of the attempts that have been made to isolate *M. leprae in vitro*, the main areas that might be important for success are considered, in light of perceived ideas of *M. leprae*'s *in vitro* characteristics.

Bacterial cytology

M. leprae has proved to be very elusive—indeed it was only finally established as a mycobacterium in 1974[5]. There is still a lack of information about its exact characteristics which has complicated the process of identification of cultures claimed to be *M. leprae*. The strains of bacilli isolated from lepromatous tissue by different workers can be grouped according to Rogers and Muir's classification of 1975[6]:

1. **Diphtheroids or Streptothrix strains** (Kedrowski[7], Delville[3], Chatterjee[3], and Skinsnes[8]). These are pleomorphic, grow easily on ordinary culture media, and are labile with respect to acid-fastness.

2. **Chromogenic acid-fast bacilli** (Clegg[9], Duval[7], Yoshie[6], Skinsnes[8], and Kato[10]). Most of the claims for the successful cultivation of *M. leprae* have been with bacilli belonging to this group. Their biological characteristics are compatible with those of spontaneously occurring non-pathogenic mycobacteria. After the primary culture, which is difficult to obtain, subcultures yield rapid-growing, pigmented colonies of pleomorphic bacilli with varying acid fastness.

3. **Non-chromogenic acid-fast bacilli** (Duval[11], Wellman[7]). These have been described by a few workers but the cultures have not survived. They have constant growth features, being moist-growing, non-chromogenic bacilli, resembling tinctorially the tubercle bacillus and morphologically the diphtheria bacillus. They multiply slowly and only on special media.

4. **Anerobic bacilli.** Miscellaneous.

Advances in identification

The mycobacteria can be divided into two subgenera on the basis of whether they are fast or slow growing. The species differ with

---

[1] This review was written in 1981 by Caroline J. Baker, B.Sc., while a fourth-year medical student at Middlesex Hospital, University of London, England. It was written in response to the annual competition set up by the British Leprosy Relief Association (LEPRA) for essays on various aspects of leprosy, and was the first prize winner for 1981. We take great pleasure in publishing this review. Miss Baker's present address is: The House at the End, Beeches Drive, Farnham Common, Slough, Berks SL2 3JT, England.

[2] Hansen, G. A. Spedalskhedens arsager. [Causes of leprosy.] Reprinted in Int. J. Lepr. **23** (1955) 307–309.

[3] Chatterjee, B. R. A non-acid-fast coccoid precursor; possible cultivable phase of *M. leprae*. Lepr. India **48** (1976) 398–405.

[4] Kirchheimer, W. H. and Storrs, E. E. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. 1. Report of lepromatoid leprosy in an experimentally infected armadillo. Int. J. Lepr. **39** (1971) 693–702.

[5] Etemade, A. H. and Convit, J. Mycolic acids from non-cultivable mycobacteria. Infect. Immun. **10** (1974) 236–239.

[6] Yoshie, Y. Advances in the microbiology of *M. leprae* in the past century. Int. J. Lepr. **41** (1973) 361–371.

[7] Duval, C. W. and Wellman, C. A critical study of the organisms cultivated from the lesions of human leprosy, with a consideration of their aetiological significance. J. Cutan. Dis. **30** (1912) 397–409.

[8] Skinsnes, O. K., Matsuo, E., Chang, P. H. C. and Anderson, B. *In vitro* cultivation of leprosy bacilli on hyaluronic acid based medium. 1. Preliminary report. Int. J. Lepr. **43** (1975) 193–203.

[9] Clegg, M. T. Some experiments on the cultivation of bacillus leprae. Phillipine J. Sci. **4** (1909) 77–79.

[10] Kato, L. The Janus-face of *Mycobacterium leprae*: Characteristics of *in vitro* grown *M. leprae* are not predictable. (Editorial) Int. J. Lepr. **45** (1977) 175–182.

[11] Duval, C. W. The cultivation of the leprosy bacillus and the experimental production of leprosy in the Japanese dancing mouse. J. Exp. Med. **12** (1910) 649–665.

respect to their phenetic, antigenic, and metabolic characteristics. Many criteria have been developed to which cultivated *M. leprae* might be expected to conform. Any method is dependent on the limited supply of infected tissue available and, therefore, must be applicable to small quantities of impure material. Since the criteria have been proposed by the same workers who are attempting to cultivate *M. leprae,* the validity of the techniques is disputed as often as the bacilli they identify.

Any technique should supplement direct inspection of any organisms by microscopy. The techniques most often applied[12, 13] are:

1. **Absence of growth on ordinary mycobacteria media.**
2. **Mouse foot pad inoculations**[14]. Contaminants, however, may produce false positives.
3. **Armadillo inoculations**[4, 15]. Both animal techniques require six or more months and, therefore, are impractical except as a final check.
4. **DOPA oxidase test**[16, 17]. Some workers, however, argue that what is being measured may be auto-oxidation.
5. **Pyridine extraction of acid fastness.** It is not known, however, whether the quality of acid fastness changes between *in vivo* and *in vitro.*
6. **Lepromin tests.** These require enough bacilli to make multiple tests and may give false crossreactions.
7. **Immunologic identity determination**[18].
8. **Antigen immunodiffusion analysis**[19].

Potentially, techniques 7 and 8 could provide a reliable positive identification, free of interference due to growth alterations occasioned by transfer to *in vitro* culture. Stanford found that the surface antigens common to all mycobacteria were present but that there was a lack of antigens characteristic of fast- or slow-growing species[19]. This distinguishes it from, e.g., *M. scrofulaceum*[20] and *M. duvalii*[7] which are both fast-growing species, but also from *M. tuberculosis* and *M. kansasii* (slow-growing species), despite its long replication time *in vivo.* In fact, it more closely resembles the *M. vaccae* group of atypical mycobacteria. It has now been found to possess a mycoside with a unique oligosaccharide segment; this could provide a very precise biochemical method for assessing the identity of a cultivated bacillus, even in very small amounts[21].

Consequences of adaptation to enable survival *in vitro*

Some workers, e.g., Chatterjee[22], have proposed that *M. leprae* has been cultivated already, but has been overlooked because it does not comply with the preconceived ideas about its characteristics. These have been published following workshops at successive International Leprosy Congresses. In fact some workers, e.g., Kato[10] and Skinsnes[23], have compared the perceived characteristics of *in vitro M. leprae* with the *in vivo* bacillus.

Kato now suggests that *M. leprae* might be a different biological entity on culture media[10], presenting different metabolic, antigenic, and pathogenic characteristics from

---

[12] Report of Workshop on Cultivation of *M. leprae.* Int. J. Lepr. **36** (1968) 559.

[13] Skinsnes, O. K. Problems in identifying *M. leprae.* (Editorial) Int. J. Lepr. **43** (1975) 267–269.

[14] Shepard, C. C. The experimental disease that follows the injection of human leprosy bacilli into foot pads of mice. J. Exp. Med. **112** (1960) 445–454.

[15] Storrs, E. E. The nine-banded armadillo: A model for leprosy and other biomedical research. Int. J. Lepr. **39** (1971) 703–714.

[16] Prabhakaran, K. Phenoloxidase of *M. leprae.* Nature **215** (1967) 436–437.

[17] Prabhakaran, K. DOPA oxidation of *M. leprae* and Binding of C-labelled DOPA by *M. leprae in vitro.* (Two Letters to the Editor) Int. J. Lepr. **45** (1977) 185–186 and 187.

[18] Matsuo, E. and Skinsnes, O. K. Specific direct fluorescent antibody identification of *Mycobacterium leprae.* Int. J. Lepr. **43** (1975) 204–209.

[19] Stanford, J. L., Rook, G. A. W., Convit, J., Godal, T., Kronvall, G., Rees, R. J. W., and Walsh, G. P. Preliminary taxonomic studies on the leprosy bacillus. Br. J. Exp. Pathol. **56** (1975) 579–585.

[20] Kato, L. *In vitro* grown *Mycobacterium leprae* probably a member of the *Mycobacterium scrofulaceum* species. (Letter to the Editor) Int. J. Lepr. **44** (1976) 385–386.

[21] Hunter, S. W. and Brennan, P. J. A novel phenolic glycolipid from *M. leprae* possibly involved in immunogenicity and pathogenicity. J. Bacteriol. **147** (1981) 728–735.

[22] Chatterjee, B. R. Has *M. leprae* been cultured already? (Letter to the Editor) Int. J. Lepr. **45** (1977) 294–297.

[23] Skinsnes, O. K., Kuba, B. A., Chang, P. H. C. and Kuwahara, T. *In vitro* cultivation of leprosy bacilli in hyaluronic acid based medium. 2. Progress and developing concept of the role of hyaluronic acid suggested by culture and armadillo infection studies. Int. J. Lepr. **46** (1978) 394–413.

the *in vivo* organism. This is feasible when it is considered that only 15%–20% of the bacterial genome is transcribed in a given situation. It has been demonstrated that a large number of genes coding for the synthesis of enzymes, certain structural proteins, and other cell components are under the control of regulatory genes whose activity depends partly on the composition of the surrounding medium. Therefore, a changed environment, in providing new activators and inhibitors of regulatory genes, will bring about the expression of many hitherto unexpressed genes.

Comparison with observations of other mycobacteria. Many workers would agree with the hypothesis that pathogenic mycobacteria growing *in vivo* may differ metabolically from the same strain grown *in vitro*[3, 24]. It has also been observed that parasitic bacteria are more fastidious in their nutritional requirements, and the mycobacteria oxidize a range of substrates which decreases as acid fastness and pathogenicity increase, e.g., enhancement of respiratory response becomes more limited from saprophytic species through *M. tuberculosis* to *M. leprae*[25]. Thus it might be that *M. leprae* is so highly adapted to an intracellular existence that, so far, the changes that have been demanded between the growth requirements *in vivo* and *in vitro* have been too great for sufficient bacilli to survive and replicate.

An interesting exception is *M. lepraemurium,* the agent of murine leprosy. This mycobacterium, identified in 1903, was also considered to be an obligate intracellular parasite, and it was used by many investigators as an interim model in studies of *M. leprae*[26, 27]. The problems incurred in the search for a successful medium were very similar to those with *M. leprae* and presumably it was equally possible that the *in vivo* and *in vitro* forms might differ. However, in the past decade reproducible cultures have been achieved and the *in vitro* organisms have almost the same biochemical and morphological characteristics as the *in vivo* bacillus[28]; moreover, they retain their pathogenicity for mice, producing characteristic murine leprosy.

In fact, since their original comparison, more differences between *M. leprae* and *M. lepraemurium* have become apparent and it is probably more useful to consider the problems of cultivation of *M. leprae* as being unique. *M. leprae*'s existence in humans is without parallel in that even transfer from host to host is virtually cell to cell. There is no known, and epidemiological evidence argues against their being an intermediate host or environmental period of recuperation from the effects of intracellular habitation. It is only successful intracellularly where there is minimal cell-mediated immunity (lepromatous leprosy), and these patients still have high levels of antibody which would opsinize any bacilli that dared to chance an extracellular existence. Thus it can only survive as a strictly intracellular form (obligate intracellular parasite), which must mean that within the human host there is very little variation in phenotype.

In contrast, several strains of *M. leprae* isolated by Chatterjee from mice inoculated with human lepromatous material, when observed after attempts to grow them *in vitro,* showed good elongation (thought to reflect active growth); whereas none was observed with bacilli direct from human lepromatous material[27].

Furthermore, in the armadillo there is evidence of extracellular microcolonies[4]. These are supposed to arise from intracellular colonies which, on dissolution of infected macrophages, are too large to be rephagocytosed and so continue to grow extracellularly in the presence of intercellular constituents. It is thought that the armadillo has weaker immune responses than humans, and it is possible that the bacilli are less debilitated, enabling them to adapt and survive extracellularly. In light of this,

---

[24] Kanai, K. Letter to the Editor. Int. J. Lepr. **45** (1975) 184–185.

[25] Segal, W. and Bloch, H. Biochemical differentiation of *M. tuberculosis* grown *in vivo* and *in vitro.* J. Bacteriol. **72** (1956) 132–141.

[26] Dhople, A. M. and Hanks, J. M. *In vivo* growth of *M. lepraemurium,* an obligate intracellular microbe. Science **197** (1977) 379–381.

[27] Hart, P. D. Further analysis of the growth (elongation) phenomenon of *Mycobacterium lepraemurium in vitro,* and relevant studies with *Mycobacterium leprae.* Int. J. Lepr. **33** (1965) 504–509.

[28] Mori, T. Biochemical properties of cultivated *Mycobacterium lepraemurium.* Int. J. Lepr. **43** (1975) 210–217.

it is interesting that Skinsnes has proposed that *in vitro* cultures are most easily achieved from armadillo than from human lepromas[23], especially coupled with Kato's observations that the oxidative activities of armadillo-grown *M. leprae* indicate that they are metabolically more competent and potent than bacilli cultured from human lepromatous tissue[29].

These observations may indicate that animal-cultivated *M. leprae* shows the first stages of the adaptation necessary for *in vitro* survival. These changes could be investigated to help provide the starting stages for a continuum of environmental changes that would allow further adaptation, culminating in a form that would be reproducible and would tolerate an *in vitro* existence.

In vitro isolation and cultivation

General considerations. Procedures aiming to isolate and cultivate *M. leprae in vitro* must consider every variable. The first problem is collection and handling of specimens, since faulty technique often leads to failure to cultivate. Optimally, specimens are taken before treatment and from the site where the organism is most likely to be.

Other important factors include stage of disease, large enough specimen, sterile container, and prompt delivery.

At the IX Workshop on Cultivation of *M. leprae* (1968)[12], it was agreed that lepromas from untreated lepromatous patients provide the most reliable source of inocula for cultivation trials. When feasible, because of problems with contamination and transport, infected, thymectomized, irradiated mice provide a more readily available source of inocula. The importance of inhibitors of microbial growth and factors toxic to tissue culture cells that may be present in the host tissues was emphasized.

Bacilli are found predominately in smears and scrapings from the skin (the corium rather than the epidermis[30]), and mucous membranes (particularly the nasal septum) of patients with nodular lepromatous leprosy. Bacilli are rare or absent in tuberculoid or borderline lesions.

Kato found that the delay between biopsy and starting a culture decreased the chances of success[29]. His bacilli were from lepromas shipped without refrigeration, the main consensus of opinion being that freezing might alter subsequent activity of the bacilli. Decontamination must always prove a problem since many specimens are obtained in primitive environments. Many of the leprosy patients will also be infected with other microorganisms[31]. One method for killing surface contaminants is to flame the tissue[11], which may also kill the bacilli.

The method of tissue preparation must be examined. Typically the methods used involve a) grinding of the tissue to a fine suspension; b) sterilization of culture media by autoclave; c) filter sterilization of serum; d) centrifugation of donor *M. leprae* suspension, followed by homogenization, incubation, and washing of the sediment; e) inoculation onto the medium; f) incubation, and g) assessment of growth by appearance and consistency of colonies on the surface and by microscopic examination of stained preparations.

One problem suggested by Pares[32], who supports the hypothesis of there being a dimorphic life cycle, is that filtration may remove any bacilli in the active (unrecognized) phase. Another major problem arises from the generally accepted fact that a heavy inoculum is required to achieve any growth. Thus there may be a carry-over of host factors in the primary culture, which may initially help promote growth, only to fail once the host factors are exhausted. This may well explain some of the results obtained using tissue or organ culture[11, 31, 33, 34].

---

[29] Kato, L. and Ishaque, M. *In vitro* cultivation of mycobacteria from human lepromas and from an armadillo infected with *Mycobacterium leprae*. Int. J. Lepr. **45** (1977) 107–113.

[30] Tomson, S. and Schwarz, J. Cultivating *M. leprae* in the dark. (Letter) Lancet **1** (1974) 169.

[31] Massalski, W. K., Shulka, R. R., Lowenstein, W. and Wierzbicki, R. Culture of Hansen's bacillus *in vitro* in a case of lepromatous leprosy. Mater. Med. Pol. **10** (1978) 35–37.

[32] Pares, Y. Comments on Dr. Chatterjee's correspondence. (Letter to the Editor) Int. J. Lepr. **46** (1978) 223–225.

[33] Biswas, S. K. Growth of *M. leprae* in thyroxine-treated culture medium—a preliminary report. Lepr. India **50** (1978) 57–63.

[34] Shukla, R. R., Massalski, W. K. and Lowenstein, W. Culture of *M. leprae* with help of desiccated thyroid. Lancet **2** (1976) 363–364.

Cross contamination. All workers have become paranoid about decontamination due to the repeated growth of similar mycobacterial species in the history of cultivation attempts. In most cases, these have subsequently been identified as distinct species and therefore have been rejected as contaminants. Many workers consistently isolate chromogenic mycobacteria when attempting to grow *M. leprae*. These organisms have had many similarities. Some workers feel that such consistent cross-contamination is very unlikely[7, 10], and it may be that chromogenic species grow symbiotically with other mycobacteria, including *M. leprae*. On the other hand, it seems paradoxical that strains growing so easily once established are so reluctant to grow in the initial cultures.

Although one suspects that there is a whole spectrum of contaminants, there are a surprising number of very similar species, notably *M. duvalii* which was only differentiated in 1974[35] and *M. vaccae* which was reported as having the same antigenic structure as *in vivo M. leprae* as recently as 1976[36]. However, so many species have been isolated from lepromas that it seems naive to believe one of these chromogens is the *in vitro M. leprae*, especially since they are also isolated from other sources[37] which presumably are not considered to be undiagnosed cases of leprosy. It may be that the frequent confusion just reflects the lack of information regarding the exact characteristics of *in vitro M. leprae*.

Cultivation trials in cell-free media. For the cultivation of an "obligate intracellular parasite," the choice of media might be expected to pose special problems, especially since other mycobacteria will also only grow on special media.

The proper preparation and selection of a medium requires systematic consideration of all variables including essential nutrients in the proper concentration, adequate salt, adequate water, freedom from inhibitory substances, proper consistency, proper reaction, sterility, temperature, and oxygenation.

In 1954 Hanks[38] made a systematic investigation of the biochemical properties and metabolic capabilities of *M. leprae*. He concluded that *M. leprae* was metabolically deficient. More recently, more sophisticated techniques have shown that host-grown leprosy bacilli are metabolically competent[39].

Every prospective cultivator has investigated the various aspects they see as critical, and some conflicting findings have emerged. Duval, for example, found that moisture and adequate illumination were both critical[11]; whereas Tomson and Schwarz advocated cultivating in the dark[30]. Duval established the optimum temperature at between 32°C and 35°C, with no growth at 37°C. On the other hand, this cannot be an absolute requirement because leprosy bacilli can be isolated from the organs (spleen, liver) removed from leprosy patients at autopsy[11]. In addition, the failure to find a poikilotherm that can support the disease is surprising, since these animals can exist at an appropriately low body temperature. Possibly their intracellular metabolism is unsuitable and therefore any differences may indicate inhibitory factors to be eliminated.

All mycobacteria require organic material for growth *in vitro*. Suitable substrates investigated for *M. leprae* include hyaluronic acid (Skinsnes[8]) and cholesterol (Kato[40]). It is possible that either may contribute to *M. leprae*'s ability to survive in macrophages *in vivo* since these cells contain considerable amounts of both. Equally, either might facilitate extracellular survival of the bacilli.

The pH of the medium must be important. Skinsnes predicted an acid optimum pH[8]. The pH of macrophages has been as-

---

[35] Godal, T., Myrvang, B., Stanford, J. L. and Samuel, D. R. Recent advances in the immunology of leprosy with special reference to new approaches in immunoprophylaxis. Bull. Inst. Pasteur **72** (1974) 273–310.

[36] Stanford, J. L. and Rook, G. A. W. Taxonomic studies on the leprosy bacillus. Int. J. Lepr. **44** (1976) 216–221.

[37] Tsukamura, M. Letter to the Editor. Int. J. Lepr. **45** (1977) 185.

[38] Hanks, J. H. Implications of Suter's review of intracellular parasites with respect to the problem of leprosy. Int. J. Lepr. **22** (1954) 12–15.

[39] Ishaque, M., Kato, L. and Skinsnes, O. K. Cytochrome-linked respiration in host grown *M. leprae* isolated from an armadillo (*Dasypus novemcinctus*, L.). Int. J. Lepr. **45** (1977) 114–119.

[40] Kato, L. Cholesterol, a factor which is required for growth of mycobacteria from leprous tissues. Int. J. Lepr. **46** (1978) 133–143.

sessed but it is difficult to know whether the relevant intracellular pH is that of the cytoplasm or the phagolysosome.

Olitzki and Levy[41] believe that they can maintain *M. leprae* for at least five months on their media by eliminating various factors that inhibit growth. The problem with this and any model investigating *in vitro* growth is that one must begin with a presumption of growth in order to demonstrate any differences.

Logically, it must be possible to manipulate substrates to achieve the desired growth, e.g., Duval[7] found that the pigment of his rapidly growing strains depended on the substrates used. Since it appears that *M. leprae* is metabolically competent, investigators need to explore the reasons why it does not realize its oxidizing potential *in vitro*. There must be a factor, e.g., lepromatous tissue, that mouse foot pads and the armadillo have in common. One possibility considered is that by washing the bacilli free of host components their metabolic capabilities are reduced due to leakiness of the bacillus' cell wall. But, if this were the sole factor, tissue culture might be expected to provide a means for culture; whereas although various cell lines and strains have been investigated, at best there has been some bacillary elongation, e.g., in Schwannoma cells, fibroblasts from lepromatous tissue and mouse peritoneal macrophages[39]. These failures may reflect the fact that an optimum environment is critical since waste products possibly inhibitory to growth will not be removed as efficiently in tissue cultures as in a live host with elaborate homeostatic mechanisms.

Discussion

In the search for a method for cultivating *M. leprae in vitro,* large numbers of acid-fast bacilli have been isolated, by far the most common being chromogenic, rapid-growing mycobacteria. Whatever their significance, the nature of these organisms does not accord with ideas of *M. leprae* being a highly specialized parasite.

Methods for characterizing *M. leprae* have become increasingly refined. Unfortunately, all criteria for identification of *in vitro* cultures are based on projections from its *in vivo* properties; whereas the *in vitro* bacillus may present apparent differences due to the fact that established test reactions may be partly due to reactions with contaminants. One example of such a difference is illustrated by *M. tuberculosis,* which has a greater immunizing capacity and decreased pathogenicity *in vitro* than the same strain *in vivo*[25]. The more recent techniques of antigen immunodiffusion analysis and immunologic identity determination reflect a more useful approach because these must remain constant independently of any phenetic changes.

A dimorphic life cycle has been hypothesized to explain the various forms of bacilli in lepromatous tissue. A series of tissue specimens and cultures could be followed to establish what changes occur to the morphological and/or biochemical properties of the bacilli with time. It is thought that the ability to produce resistant, acid-fast, coccobacillary forms is significant to its survival and transmission *in vivo. In vitro* survival would require an inoculum containing enough actively dividing bacilli for growth to become established. This might involve a long initial latent period when the bacilli would be vulnerable to inhibition by more rapidly growing contaminants. Care must be taken to ensure that the system does not deteriorate during this period. A successful *in vitro* culture may contain a mixture of organisms which might not all conform to the idea of *M. leprae* as an acid-fast bacillus. These forms must be recognized when devising methods for removing contaminants which may also be very similar morphologically and generically.

The theory of dimorphism is distinct from claims that the *in vitro* leprosy bacillus is a member of the *M. scrofulaceum* species[20]. This hypothesis is incredible when one considers the large number of scotochromogenic species that have been isolated.

It has been hypothesized that leprosy is a recent disease[19] since there is no evidence of its existing prior to 1000 B.C. It is therefore possible that it was derived from a still existing progenitor which may be an environmental saprophyte. Its "young" nature, coupled with the exacting requirements of intracellular existence, may prevent it from

[41] Olitzki, A. L. and Levy, L. Growth inhibitors and promotors acting on *M. leprae.* Isr. J. Med. Sci. **8** (1972) 1940–1942.

developing as a distinct entity *in vitro.* It may be that the chromogenic species, which themselves show considerable heterogeneity[20], are closely related to, or are the progenitor, existing symbiotically with *M. leprae,* which is the dominant species *in vivo.* When transferred to an *in vitro* situation, the chromogenic species is better adapted and becomes established as the dominant species. Thus a medium which specifically suppressed the chromogen would be needed, e.g., based on antigenic identity. This must be possible since the chromogens grown vary between workers.

Accepting that *M. leprae* is highly adapted to intracellular existence, the intracellular requirement cannot be absolute since extracellular colonies have been observed in the armadillo. Therefore, investigations as to why the bacilli survive may provide a clue as to the next step in devising an *in vitro* cultivation system.

—Caroline J. Baker, B.Sc.

The House at the End
Beeches Drive
Farnham Common
Slough, Berks SL2 3JT
England

Acknowledgment. The author wishes to thank Dr. A. C. McDougall for the encouragement and assistance given during the revision of this essay.