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EDITORIALS

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The Janus-Face of *Mycobacterium leprae*: Characteristics of *In Vitro* Grown *M. leprae* Are Not Predictable

"It would be natural to predict that the same species of microorganisms takes different ways of living depending upon different in vitro *and* in vivo *environment."*
(Koomi Kanai and Eiko Kondo. Jap. J. Med. Sci. Biol. **27** *[1974] 135-160)*

It is not the objective of the present exercise to defend any culture of mycobacteria described by others or ourselves as the etiologic agent of leprosy. Many cultivators cultivated the so far "non-cultivable" *M. leprae,* but no cultivator ever produced a culture which the competing cultivators accepted or the critics recognized as the authentic *in vitro M. leprae.* Who is to blame, the cultivator, the competitor or the critic? Philosophically, all three of them confront the same problem which is insurmountable until one reconsiders the parameters and norms according to which the *in vitro* cultured *M. leprae* is recognizable. I do not claim to have the answer. I have no data at hand to draw the metabolic map of *in vitro* grown *M. leprae,* but as a prospective cultivator I simply want to continue the dialogue which started 103 years ago. Since the cultivation of the leprosy bacillus remains the top priority in leprosy research, it is time to reconsider our views of the characteristics of *in vitro* grown *M. leprae.* It is time to open a new avenue in our philosophy because if we do not admit our errors, misbeliefs and misconceptions regarding *M. leprae,* our culture media will remain sterile for the next century.

We drew up the image of the most wanted *in vitro M. leprae* as it existed in our imagination. I propose to forget about the guidelines which we authoritatively set since time will show that these were wrong. *M. leprae,* this most elusive microorganism, must have most unusual and unexpected *in vitro* characteristics, which probably did not even exist in our imagination. Any categorical prediction of these characteristics may lead cultivation trials into a dead end. Predicting the image of *in vitro M. leprae* is as illogical as

drawing up the physiognomy of a person whom you never met before but went to pick up at the airport.

Many of us dared to describe the anatomy of *in vitro* grown *M. leprae*. I admit that I often regretted subscribing to the predictions. I am not asking others to withdraw, just to reconsider and for all pragmatic purposes to reason according to basic laws of the philosophy of science. Then I will suggest two pragmatic steps before rejecting or accepting any culture to be identical to the etiologic agent of leprosy. Recently a great deal of data has been accumulated on *M. leprae* and in the light of this newly acquired knowledge it seems that the "two faces" of this bacillus are not identical, such as was the case of Janus. *M. leprae* might be a different biological entity inside the host cell and on culture media, with different metabolism, antigenicity and pathogenicity.

In the times of Romulus, Servio and Silla, the augurs and auspices predicted the future of emperors, the outcome of wars, the color of eyes, hair and skin and the sex of children still unborn. The haruspices,[1] also recognized experts at workshops of successive International Congresses, predicted the characteristics of *M. leprae* to be cultured in a test tube. The predictions were made on the unfounded assumption that the leprosy bacillus will behave in the test tube exactly the same way as in the human macrophage. The haruspices, often without much knowledge of bacteriology, made these declarations *ex cathedra* by simply looking into the viscera of the dead macrophages. Bacilli obtained from these cells were noncultivable on Lowenstein or Dubos media, they produced disease in the armadillo, divided a few times in the foot pads of mice, produced the lepromin reaction in tuberculoid patients and provoked no granuloma in the lepromatous. The bacilli even gave a positive color test with the most unstable DOPA. Such is the profile of *M. leprae* in the macrophage and, therefore, such must be its characteristics in the test tube. For every student of science philosophy, this is one of the most unfounded and unscientific predictions scientists ever produced.

Cato recorded that when the augurs met they could not avoid laughing. "*Augures se videre non possunt sine rire.*" They know they were wrong but no harm was done. Not so with the leprosy bacillus. Based on limited knowledge of the characteristics of host grown *M. leprae* how could we, haruspices of leprology, dare to predict the biochemical, physiologic and immunologic characteristics of *M. leprae* once cultured on artificial culture media. Such predictions, made at several International Congresses, are dangerous and contrary to the interest of the patient, science and scientists (in this case the cultivators).

It would be interesting to write the comprehensive history of the cultivation of *M. leprae* from the time of its discovery until today. The comprehensive history will probably never be written since dried-out cultures cannot talk and most investigators did not write down on paper their deceptions. Often it was obvious that the cultures obtained were banal laboratory contaminants rather than *M. leprae*. However, in several instances it happened that the investigator thought that he had succeeded in culturing that "obligate intracellular parasite," but soon he had to learn or was told by others that the culture was, though a mycobacterium, not related to *M. leprae*. Whenever a claim of cultivation was discredited, the verdict was based on the assumption that the culture must have the same characteristics as the *in vivo* grown *M. leprae*. Since these criteria were not fulfilled, the cultures dried out on the shelves and the cultivator sometimes left the workshop without leaving his new address behind. Other investigators who produced some kind of a culture learned surprisingly that others had produced a very similar kind of culture half a century before, and thus it did not take long for this culture to be shelved and discredited. However, it is most instructive to make a close examination over the inventory of the available mycobacteria cultured from leprous tissue. Interestingly enough, most of them were slow growers requiring sometimes a very long period of time to obtain the primary culture. They sooner or later produced pigments in the presence or absence of light and they sooner or later grew on conventional media de-

[1] Haruspices = In ancient Rome, one of a minor class of priests who practiced divination. The allusion here refers to the presupposition of presumptive "facts" before their actual determination, perhaps because the means for their determination have not been available.

signed for mycobacteria. Either retrospectively or actually, the cultures available were identified as being scotochromogenic and belonging to the group of scrofulacea.

Those on the other side of the fence might say that this is a coincidence and/or that this happens because the scrofulacea species is widely ubiquitous. The prejudiced reader is conditioned to misconceptions such as the concept that *M. leprae* is "noncultivable," that it is "an obligate intracellular parasite" and, he accepts the verdict without asking further questions. The truth, however, is that the scrofulacea are not so widely dispersed in nature as one may think. In clinical and public health laboratories, where hundreds of cultures of different types of mycobacteria are handled, stored, cultured and transferred and where hundreds of clinical specimens arrive daily for bacteriologic identification and diagnosis, one never confronts the problem of secondary contamination of cultures or clinical specimens with scrofulacea. Before writing down this statement, I inquired of ten trained bacteriologists, directors of ten reputable bacteriologic laboratories where more than 10,000 specimens are examined yearly for the diagnosis of mycobacteria. To my great surprise, none of them ever heard of a cross contamination with scrofulacea or of scrofulacea as a wild contaminant in specimens or cultures. They all agreed though that *M. scrofulaceum* and related cultures are often isolated from different specimens. I was also told what I knew before, namely that other mycobacteria such as *M. smegmatis, phlei, aquae* and soil mycobacteria are more ubiquitous and ubiquitous in soil, water, skin, laboratory shelves, incubators and in organs of laboratory animals. A question thus arises. Is it really a coincidence or is it because of the wide distribution of scrofulacea in nature that such bacteria were isolated, cultured and identified so often from leprous tissue?

Without analyzing in any great detail the available literature, personal communications and unpublished data, it is worthwhile to examine the history of such scotochromogenic slow growing cultures which made headlines or raised some hope in the history of the cultivation of *M. leprae*. The list of reported cultures is impressive. I selected six out of the many scotochromogenic cultures obtained because their cultivation and characteristics are described in detail, sometimes as colorfully as is deserved by pigment forming strains of mycobacteria. The reports selected are those of Clegg in 1909, Duval in 1910, Walker in 1923, Sister Marie-Suzanne in 1953, of the Skinsnes group in 1975, and of ourselves in 1976. The first five authors claimed the successful cultivation of the leprosy bacillus; our group simply stated that pigment forming mycobacteria are regularly cultured from human, armadillo and rat leprous tissue.

May I repeat again that I do not want to analyze whether the claims are right or wrong or whether the cultivation of *M. leprae* was ever achieved, but I wish to carry further the dialogue, to ask for comments and to give warning signals and urge the reorientation of our list of guidelines on *sine qua non* characteristics of *in vitro* grown *M. leprae*.

The leprosy bacilli of Clegg (Philipp. J. Sci., 1909, **4:** 403-415). "The acid-fast organisms had been obtained in symbiosis with amoebae from the spleen in two cases and from skin nodules in three other cases of leprosy. Similar cultures have been obtained from leprous tissue in three additional cases". In Clegg's cultures, acid-fast bacilli did multiply but they differed morphologically from the host-grown leprosy bacillus. Clegg was not surprised that the strongly acid-fast bacilli were unusually short and often coccoid and he stated: "We know nothing regarding the morphology of bacillus leprae on artificial media." He discussed carefully the possible sources of error, included all kinds of controls, and presented conclusive evidence that the bacilli originated from the leprous tissue. Only the primary cultures were difficult to obtain; thereafter the bacilli were easily subcultured on different kinds of simple bacteriologic culture media and the bacilli formed a yellowish or orange pigment when growing on the surface of the media. The cultures were slow growers, scotochromogenic and belonged most probably to the scrofulacea species. In animals inoculated with the bacilli, some lesions occurred from which bacilli could not be cultured on conventional media on which other mycobacteria grow abundantly. Ernest Linwood Walker (Am. J. Trop. Med. 1923, **3:** 417-424) pointed out that, "The chromogenic acid-fast bacilli of Clegg have been isolated repeatedly from leprous

lesions by competent bacteriologists working in different parts of the world. The bacilli which Clegg isolated at Manilla, Philippine Islands, were grown on a medium which is of very low nutrient value. The essential factor in the techniques of Clegg is the symbiotic amoebae which provide a condition of growth for the lepra bacillus somewhat approaching the intracellular parasitism in the tissues. Clegg calls attention to 'the difficulty of obtaining the initial growth of this acid-fast bacillus, in that of a large number of cultures made from the same material containing innumerable lepra bacilli, only a few will show growth of the acid-fast organism.'"

Clegg continued his experiments at the Leprosy Investigation Station of the United States Public Health Services in Hawaii. With his collaborators and successors, Currie, Brinckerhoff and Hollman (U.S. Publ. Health Reports, 1910: 1173), Currie, Clegg and Hollman (U.S. Publ. Health Bull. 1911, No. 47) and McCoy (U.S. Publ. Health Bull. 1914, No. 47) cultivated the same type of pigment forming chromogenic acid-fast bacilli regularly from different cases of leprosy and studied the pure cultures in detail.

The chromogenic acid-fast bacilli of Duval (J. Exp. Med. 1910, **12:** 649-665). Already in 1910, Duval confirmed the findings of Clegg and he cultured chromogenic acid-fast bacilli from leprous lesions in symbiosis with amoebae. He proposed that the symbiotic cells break down proteins to simple units which leprosy bacilli could utilize. He prepared various types of media consisting of trypsin digested proteins or composition of different amino acids. On these media, he again cultivated successfully chromogenic acid-fast bacilli from leprosy cases.

Duval also pointed out repeatedly that the initial multiplication of the leprosy bacilli outside of the animal body is obtained with extraordinary difficulty or not at all unless special media and methods are employed. Duval was not surprised "since other pathogenic microorganisms behave in a similar manner. Certain strains of the tubercle family which at first grow feebly, and in some instances not at all on the most preferable artificial medium." Duval further states that, "the fact that the initial growth of the leprosy bacillus is so difficult to obtain, even in the presence of special foodstuffs, although once cultivated it grows profusely on ordinary media, is not surprising when we consider that the bacillus tuberculosis is incapable of cultivation directly from the tissues on glycerin agar, upon which medium it grows well with subsequent generations. Rapid growth of bacillus lepra . . . is a property quickly acquired by cultures that are no longer influenced by the conditions of the host." In the host, the bacilli, "multiply very slowly and are long, slender." In the culture medium, the bacilli grow, "with great rapidity and are short, almost coccoid." The continued growth of bacillus leprae is not insured, "until the culture is accustomed to the new environment." As a method of cultivation, Duval points out that it is important that the medium is rich in trypsin digested protein and glycerol. Cysteine and tryptophan, "serve to start multiplication of lepra bacilli." Again and again Duval mentions the potent metabolic competency of the leprosy bacilli. He points out that these cells can adapt to and grow on any and most simple substrates. "Nutrient agar or any of the ordinary laboratory media will not serve as a transfer medium until the cultures have become accustomed to artificial conditions. Bacillus leprae will also grow on the various blood agar media once they are accustomed to artificial conditions." Then Duval observes the same kind of pigment formation which Clegg already described. "It is noteworthy that the growth in the tissues and in the first dozen or so generations of artificial media is entirely without pigment." However, "the chromogenic property of lepra culture is a constant and characteristic feature for rapidly growing strains." The pigment formation depends on the substrates used. "In the absence of peptone, the colonies are faint lemon-yellow, in the presence of peptides the colonies are deep orange. Growth of the leprosy bacillus occurs only in the presence of products of trypsin digestion." He further states that without the amino acid in the medium, "the primary multiplication of the leprosy bacilli cannot be secured."

Only a few investigators tried to verify the results of Duval with a consideration of their etiologic significance (J. Infect. Dis. 1912, **2:** 116-139). The rapidly growing scotochromogenic organisms are classified as a separate entity by Stanford and Gunthorpe (Br. J. Exp. Pathol. 1971, **52:** 627-637) as *M. duvalii* (new species). Four strains of *M. duvalii*

are known at present and all of them originate from cases of lepromatous leprosy. *M. duvalii* is not an environmental mycobacterium and Godal, Myrvang, Stanford and Samuel described a striking antigenic similarity between *M. duvalii* and *M. leprae* (Bull. Inst. Pasteur, 1974, **72:** 273-310). The obtained results suggested the possibility of, "inducing protective immunity to leprosy by a combination of BCG plus *M. duvalii,* . . . particularly since BCG alone has been found to induce some protection against leprosy" *(ibid).*

The question thus arises as to whether *M. duvalii* is some obscure superinfectant of the leprous tissue or the Janus face of the etiologic agent of leprosy. The same philosophy applies for all other scotochromogenic scrofulacea type microorganisms isolated so regularly from leprous tissue.

The chromogenic acid-fast bacilli of Ernest Linwood Walker (Am. J. Trop. Med. 1923, **3:** 417-424). This investigator first repeated the technics of Clegg using mixed cultures of amoebae on a simple medium described by Clegg inoculated with lepra bacilli. "In a small percentage of these cultures, acid-fast bacilli developed after several weeks. When acid-fast bacilli were isolated in pure culture, they grow readily on ordinary nutrient media and produce pigments varying from yellow to orange color." Walker also succeeded in growing a series of chromogenic acid-fast bacilli in the absence of the symbiotic amoebae. Walker claims that: "Chromogenic acid-fast bacilli can be cultivated repeatedly from leprous lesions. The essential factor for the initial growth of these organisms appeared to be the lean medium which contains only traces of nutrient material. The primary growth of these chromogenic acid-fast bacillus in my experience never takes place in ordinary nutrient media: but when growth has once been established on the lean medium, it can be transplanted and will grow on ordinary nutrient media." The result, "might indicate that it is a contamination with a widely distributed saprophytic acid-fast organism," and "a possibility that it may be a contamination, cannot as yet be absolutely excluded."

The scotochromogenic bacilli of Sister Marie-Suzanne (VI. Congr. Int. Microbiol. Roma, **2** (Sect. 8-16): 655-656, 1953). The culture is known as *M. marianum* and its origin and characteristics are well documented. It is a typical scotochromogenic culture, forming yellowish to orange pigments. It was first isolated after an extremely long incubation period on leprous tissue which underwent advanced autolysis. So the "medium" was without doubt rich in peptides, amino acids and other degradation products of protein. The culture was a slow grower but was easily subcultured on conventional culture media used to grow mycobacteria. This culture was identified and classified as a scotochromogenic strain belonging to the *M. scrofulacea* species. It retained one peculiar intracellular property, namely that of being stained by Sudan black B.

The scotochromogenic leprosy bacilli of Skinsnes et al (Int. J. Lepr. 1975, **43:** 193-209). Sixty-five years after Clegg's pigmented bacilli were cultured, history repeated itself in Honolulu. On a hyaluronic acid-based medium, Skinsnes *et al* cultured from human, rat and armadillo leprous tissue a strain of mycobacteria which was claimed to be identical with *M. leprae.* The biography of the culture is similar to those of Clegg, Duval, McCoy and Sister Marie-Suzanne. It was difficult to obtain the primary culture, it grew only in very specific conditions, and it took a long time to develop.

The cultures produced by the Honolulu group were born out of a logical approach. Hyaluronic acid was incorporated in the medium because after many years of investigation the authors found that the leprosy bacillus has the enzymatic machinery to split the huge molecule in the connective tissue where the bacilli grow abundantly. Their primary culture did not grow on Lowenstein and Dubos media but after more than ten subcultures in the homologous medium, the cultures became adapted to extracellular life and then grew as scotochromogenic colonies or yellow pigmented sediment in the liquid media. Using conventional bacteriologic technics, skilled microbiologists classified the cultures as being a member of the *M. scrofulaceum* species. Their verdict was that

the cultures of the Honolulu group, "are not identical with the etiologic agent of leprosy."

The scotochromogenic cultures of Kato and Ishaque (Int. J. Lepr. 1976, **44:** 431-434; *ibid,* 1977, in press; Acta Leprol., 1977, in press). We were searching systematically for substrates which *M. leprae* and *M. lepraemurium* can oxidize to produce energy for growth and multiplication. Seven such substrates were identified during the last five years. Yeast extract was one which was consistantly oxidized by *in vivo* grown mycobacteria. When this substrate was incorporated as a prospective source of energy and nitrogen, and glycerol as a source of carbon, with sheep serum supplemented in the medium, we regularly grew pigment-forming cultures of mycobacteria from human, and armadillo leprous tissue. On 17 different occasions from 17 rat lepromas we grew without exception the same scotochromogenic acid-fast microorganisms. Careful controls and investigations could not reveal any secondary contamination by these microorganisms of our chemicals, shelves, glassware and animals. Similar cultures were obtained on the same media from lepromas received from different geographic locations as well as from different organs and subcutaneous lepromas of an armadillo. We were satisfied first to learn that the primary culture did not grow on liquid Sauton and Dubos media and on none of the solid media such as Lowenstein, Dubos and Sauton. After a few subcultures, however, each of the cultures obtained grew as slow-growing scotochromogens in Lowenstein media and were identified with conventional bacteriologic methods as belonging to the *M. scrofulaceum* species. Rifampicin and DDS inhibited the *in vitro* growth of all the cultures, but the inhibition was evident only in purely synthetic media. Other antimycobacterial agents were inactive against our cultures. The murine strains reinjected into mice produced but a limited disease. The cultures obtained from human lepromas did not multiply in the foot pad of mice and produced positive delayed reactions in the skin of tuberculoid as well as lepromatous leprosy patients. The cultures, *horribile dictu,* did not oxidize DOPA either. Therefore, according to the guidelines set by the experts, our cultures are not identical to the etiologic agent of either human or murine leprosy.

Logic, however, dictates that we might have the wrong image in our imagination on the characteristics of *in vitro* grown *M. leprae.* It has already been shown in two independent laboratories, and questioned in many others, that DOPA oxidation is not a unique characteristic of *M. leprae.* It is not *M. leprae* isolated from the host which oxidizes DOPA but this unstable chemical structure is oxidized by physical interference with connective tissue constituents. Should *in vitro* grown *M. leprae* multiply in the foot pad and produce the disease in the armadillo, when adapted to saprophytic life on a culture medium? This is definitely not a law in biology. Most strains of pathogenic tubercle bacilli and many other pathogens lose their infectivity after being adapted to culture media. Why should *M. leprae,* once cultured, remain pathogenic for the mouse foot pad or the armadillo? Basic antigenicity of these cultures remained intact since both the Honolulu strains and the Montreal, human-derived, cultures gave positive immunofluorescence reactions with anti-*M. leprae* sera. Our cultures did not produce the expected lepromin type reaction in the susceptible and resistant human skin. Why should they? The classical lepromin is prepared from human and armadillo lepromas. This starting material is denaturated by heat in a mixture of probably hundreds of constituents of the leproma together with the host grown *M. leprae.* Is it not a basic rule that host grown *M. leprae* with host tissue produces the specific lepromin reaction? While we have all reasons to expect that cultured *M. leprae* should give the same selective Mitsuda type skin reaction, this cannot be accepted as a rule. Many alterations can occur in the biologic characteristics of a living entity when adapted to a new environment. It would be repetitious to consider in more detail the expected characteristics of *in vitro* grown *M. leprae.* This was carefully analyzed in an article recently written by Skinsnes. A species, adapted to new environment and to survival in, and adaptation to, new substrates, new energy sources, new carbon sources might develop irreversible alterations. A *Sarcina* type soil microorganism which multiplies abundantly in mud, can be grown when adapted to culture media. The same culture of *Sarcina* reintroduced into the same mud will die without any sign of multiplication (Bacteriol. Rev. 1970, **34:** 82-97). It is a long

way from the cytoplasm of a macrophage into the test tube where *M. leprae* is offered yeast extract, glycerol and sheep serum or hyaluronic acid. It would be surprising if this artificially grown cell would behave in every respect the same way as during its parasitic phase of life.

It is hard to reconcile that it is a coincidence that Clegg, Duval, McCoy, Sister Marie-Suzanne, the Skinsnes group and the Montreal group cultured exactly the same type of scotochromogenic mycobacteria from leprous tissue. We have also detailed information that investigators discarded their scotochromogenic cultures, obtained from leprous tissue, simply because these were not the types of cultures they expected to be grown from leprous material. We would be grateful to the readers if they would kindly communicate with us regarding any knowledge they have, or experience they had, or any information they could provide on scotochromogenic cultures obtained from human or murine leprosy material.

It is probably not a coincidence that the authors here cited, isolated without exception, scotochromogenic *M. scrofulaceum* type mycobacteria when attempting to grow *M. leprae*. These cultures have had many similarities in their biography and characteristics. They were all cultured from biopsy and autopsy material from leprosy patients. In every case the primary culture was extremely slow and difficult to obtain and subcultures were easily adapted to most simple culture media. In every case the primary cultures contained partially or completely hydrolyzed or autolyzed protein material. These were either peptides or amino acids or autolyzed tissue, or yeast extract in the experiments of Skinsnes and our laboratory. It is a fact that whenever mycobacteria were isolated from leprous tissue it was an *M. scrofulaceum* type culture and not a *phlei*, or a *smegmatis* or soil mycobacteria, or any other member of the long list of so far identifiable mycobacteria.

I definitely subscribe to the philosophy that characteristics of *in vitro* grown *M. leprae* cannot be predicted or forecast. Accepting these predictions as guidelines for identifying any culture as the causative agent of leprosy might be most misleading. Again I must repeat that I do not presently or unequivocally accept any of the above cultures as identical to *M. leprae*, but I invite the reader to admit that we now have the faintest idea what characteristics *M. leprae* will possess once grown on artificial culture media. We have no reason to accept blindly every culture produced from leprous tissue as the etiologic agent of leprosy. Similarly it would be illogical to discredit a culture produced on media of logic just because the culture does not behave as expected and does not have the biologic, immunologic and pathogenic characteristics predicted or forecasted on the basis of inadequate and limited knowledge.

May I propose a pragmatic way to achieve cultivation. It has been repeatedly pointed out and recognized that the cultivation of the leprosy bacillus will be achieved only by gaining appropriate knowledge regarding the metabolism of the *in vivo* grown, hitherto noncultivable *M. leprae*. The knowledge thus gained will permit us to propose the formulation of logical culture media in which appropriate substrates will serve as source of energy, nitrogen, carbon and other substances for growth, multiplication and virulence. Whenever mycobacteria from human or armadillo leprous tissue are cultured regularly on such media, investigators should join efforts in trying to obtain similar cultures from as much biopsy and autopsy material as possible. If the culture medium is really the right base to which *M. leprae* can be adapted, qualified investigators should have no great difficulties in obtaining the same cultures as described by the first cultivators. The proof of the pudding is in the eating. The experiments should be repeated several times, in several laboratories, with different material and several variations.

The first step to the cultivation is to have the right culture media at hand for regular cultivation of mycobacteria from human lepromatous leprosy patients and from *M. leprae* infected armadillos. Only after this first goal is achieved should we speculate on the identity of the cultures obtained. Only then should we ask the question whether or not this is the etiologic agent of leprosy and try to obtain as much information as possible on the characteristics of the cultures obtained so as to compare them with those of *in vivo* grown *M. leprae*. On these latter characteristics, however, we need much more information than we now possess. We should in anticipation expect that the characteristics of cultures obtained will not be identical with

those of the *in vivo* grown *M. leprae* cells. With modern tools, advanced knowledge and systematic investigations we will then have the possibility of manipulating the cultures in such a way that their immunologic characteristics, pathogenicity and sensitivity to antibacterial agents will be shown to be identical or close enough in host-grown and *in vitro* grown *M. leprae*.

In conclusion, cultivators should be helped, not discouraged. Their cultures should be investigated without prejudice, keeping in mind that the characteristics of *in vitro* grown *M. leprae* cannot be predicted on the basis of our limited knowledge of the *in vivo* grown *M. leprae*. This elusive microorganism has, indeed, a Janus-face. One face is smiling at the macrophage and the other is grimacing at the culture media, or vice versa!

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