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Leprosy Review: The Bacteriology of *Mycobacterium leprae*[1]

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The study of the bacteriology of *Mycobacterium leprae*, "Hansen's bacillus," made an auspicious start. The discovery of the bacterium in about 1873 ([1]) [the exact date is uncertain since the report was not published at once ([2])] by Armauer Hansen preceded the discovery of the tubercle bacillus by a decade; indeed *M. leprae* appears to have been the first human pathogenic bacterium to be identified. The discovery was made in a country with a developed medical service, and was part of a general investigation of a serious epidemic which led to a full description of the clinical and pathological aspects of leprosy and to the recognition that it is an infectious disease.

Unfortunately, apart from descriptions of the bacteria found in leprosy lesions as observed under the light microscope, knowledge of the putative cause of the disease made little further progress until the middle of the present century and is even now absurdly inadequate. The reason is that no organism bearing a close pathological, immunological or chemical resemblance to Hansen's bacillus has ever been cultivated on an artificial medium. *M. leprae* seems to survive but not to multiply significantly in tissue culture (see below); no animal infection was convincingly demonstrated until 1965 and no infection capable of producing enough bacteria for biochemical study was available until a century after the original discovery of the organism.

Since human inventiveness is not inhibited by lack of facts, various alternative theories to the simple one, that Hansen's bacillus causes leprosy, have been devised. Of these the only theories that concern the present review are those that claim that types of microorganisms with properties different from those of Hansen's bacillus are the cause of leprosy. There have been many reports of successful cultivation of organisms from leprosy tissues. The bacteria isolated may be classified into two main categories: mycobacteria related to *M. scrofulaceum* and "coryneform" bacteria for which the name "leprosy-derived corynebacteria" (LDC) is commonly used. These two groups will be discussed below. The convention is here adopted that the formal name *Mycobacterium leprae* refers to Hansen's bacillus as orginally described by Hansen and as characterized in the eighth edition of Bergey's *Manual of Determinative Bacteriology* ([3]), and it specifically excludes the cultivable organisms referred to above. The bulk of the review will be concerned with the bacteriological properties of *M. leprae*.

MORPHOLOGY

Size and shape

The description of the bacterium in "Bergey" forms a convenient starting point for a discussion of *M. leprae*. It is a strongly acid-fast rod, 1 to 8 μm long and 0.3 μm in diameter, with parallel sides and rounded ends. These dimensions are based on light microscopic measurements; measurements with the electron microscope on thin sections suggest a diameter of 0.25 to 0.3 μm, although some cells may be morphologically aberrant and may have a larger diameter. Measurement of a small population

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of purified bacteria gave a length of 2.1 ± 0.5 (S.D.) μm [4]. Very long cells occur but are uncommon.

In infected tissues the organisms commonly occur in "clumps" or globi which may become very large, apparently containing hundreds of bacteria. In smaller clumps the individual cells occur in a parallel array to give the appearance of "bundles of cigars."

Acid fastness: the Morphological Index

The acid fastness of *M. leprae* has two unusual features: it is irregular in many, even most, of the organisms and it is removed by extraction with pyridine. The irregularity seems to be a sign of a more fundamental character of *M. leprae* in tissues, that a majority of the bacterial cells are not viable. The irregular staining corresponds to a partial loss of the contents of the cells consequent on death. This idea, which met considerable resistance when it was first suggested, was based on studies of cultivable "model" organisms [5]. It was possible to show that loss of morphological intactness and viability were correlated. It was also possible to show, by a technical *tour de force,* that the exact pattern of irregular acid-fast staining in an individual cell corresponded to the arrangement of electron-dense residual contents as seen in the electron microscope [6]. When the mouse foot pad model, the first unquestionable infection of an experimental animal by *M. leprae,* was developed [7] it was possible actually to measure the viability of the organism. There is a good correlation between the numbers of morphologically intact cells and the infectivity for mice [8, 9]. Fewer than ten morphologically intact bacteria are sufficient to establish an infection [8].

Measurement of morphological intactness forms the basis of the Morphological Index (MI) [see [10]] which is much used by leprologists to follow, for example, the progress of therapy. While it has been important, its measurement is technically demanding, requiring skilled staff and excellent microscopes. Even laboratories familiar with the technique fail to agree as to the *absolute* numbers of intact bacteria in the same suspension (Dr. R. J. W. Rees, personal communication) although there is good internal consistency within laboratories and good correlation between morphology and viability [11]. A theoretical problem is that it is quite unknown what interval elapses between biological death and loss of morphological intactness. The earlier controversy over, and the present dissatisfaction with, the MI exemplify the inconvenience of having no system in which *M. leprae* may be cultivated *in vitro.* Reliable estimates of viability must be made using experimental animals.

Pyridine extraction

Pyridine extractability of acid fastness was first noted by Fisher and Barksdale [12] and shortly afterwards confirmed [13]; treatment of smears of *M. leprae* with pyridine destroys their ability to retain the stain. This phenomenon does not apply to any other mycobacterial species, at least not unless the extraction is greatly prolonged. *M. leprae* from human and from animal sources behaves similarly [14]. The test works well only when performed with clean dry pyridine [15]; failure to observe this technical detail probably accounts for the reported failures [16, 17] to repeat the observation. In laboratories where the technique has been properly worked out it provides a convenient method for distinguishing between *M. leprae* and other mycobacteria [15]. There is some evidence that the acid fastness of mycobacteria is related to the properties of their surface layers [18], and that lipids may be involved, but understanding of the mechanism of the stain is still incomplete.

CULTIVATION *IN VIVO*

Tissue culture

Since *M. leprae* will not grow on any bacteriological medium known at present, the animal and tissue culture systems that allow multiplication to be detected have an unusual importance, over and above any information they may give about immunological or pathological processes in the human disease. Unequivocal evidence of consistent multiplication of *M. leprae* in tissue cultures has not yet been obtained. However, there is evidence of incorporation of tritium-labelled thymidine into bacteria in naturally [19] and experimentally infected cells [20], and of increase in bacterial num-

bers in macrophages ([20A, 21]). Typically these effects cannot be obtained in every culture. The most thoroughly studied system has been that of *M. leprae* phagocytosed by normal human macrophages ([22]). In this case about 70% of the cultures gave evidence of incorporation of thymidine into the bacteria. Actual amounts incorporated were highly variable, so that the method could not be used, for example, to measure numbers of viable bacteria. It seems possible that it may be useful for measuring drug sensitivity ([23]) more rapidly than in infected animals. Thymidine is chosen as a label since it is incorporated primarily into DNA. DNA synthesis is the most fundamental correlate of bacterial multiplication but scarcely occurs in macrophages themselves, so that "blanks" are usually low. Unfortunately it seems that thymidine is poorly taken up by mycobacteria (Dr. D. B. Young, personal communication), including *M. leprae,* and so its use is not a very sensitive method of measuring viability.

The mouse foot pad

Probably the most important bacteriological finding since the identification of *M. leprae* was the discovery of the mouse foot pad model ([7]) which, together with its development in immunologically impaired mice, where growth of the bacteria is more extensive ([24]), still forms the mainstay of leprosy bacteriology. In normal mice an injection of 10^4 *M. leprae* (including at least a proportion of viable bacteria) into the hind foot pad is followed by multiplication to about 10^6 organisms in about six months. No subsequent increase in bacterial numbers occurs, although morphological degeneration of the bacteria proceeds slowly; slow dissemination occurs from the site of infection ([25]). In immunologically impaired mice the local ceiling of multiplication is about 100 times higher, and more extensive dissemination to other cool sites (nose, ears) occurs. The higher ceiling is useful where numbers of viable bacteria in suspensions containing only a small proportion of viable organisms must be measured.

Apart from its use in measuring viability and sensitivity to drugs, and in studying the immunology and pathology of *M. leprae* in a controlled infection, the foot pad infection has been used to obtain basic bacteriological information about the organism. In the foot pad *M. leprae* has a mean generation time of 11 to 12 days when growing exponentially ([8, 26]) (a uniquely slow rate even among the slow-growing mycobacteria). There seem to be no variations in this rate among samples of *M. leprae* from many sources. There are, however, variations in the maximum numbers achieved and in the time needed to achieve them ([27]); these differences persist on subculture of individual strains and provide the only evidence so far of the existence of different strains of *M. leprae.* In immunologically deficient mice the fact that the sites to which the bacteria spread are "cool" sites ([28]) seems to support the clinically derived view that *M. leprae* prefers a growth temperature of somewhat less than 37°C ([29]). [Experiments with mice kept at various temperatures seem to confirm the clinical observation ([30]) but the situation must be more complicated than this: mice kept without air conditioning at ambient temperatures approaching 37°C are susceptible to the foot pad infection with *M. leprae* ([31]).] Bacteria in the foot pad grow mostly in the same habitat as they do in man, the macrophage, but are also found in nerves (also as in man) ([28]). Infection of nerves is an important, if unusual, taxonomic marker in this organism ([32]).

Bacteriology of *M. leprae* up to 1972

The four characters recommended in "Bergey" ([3]) for the identification of *M. leprae* have now been discussed: 1) acid fastness, 2) failure to grow on bacteriological media, 3) characteristic pattern of growth in the mouse foot pad, and 4) causing human leprosy. The description included an (unconfirmed) report of the presence of acid phosphatase and cytochrome oxidase and a mention of DOPA oxidase (see below) of a type apparently unique to this organism. On the whole, this is a poor foundation on which to base the definition of a bacterial species.

The nine-banded armadillo

Since 1972 a greatly increased supply of material has been available, as a result of the discovery that the nine-banded armadillo is susceptible to leprosy ([33]). This primitive mammal has a core temperature significantly lower than that of most mammals, and becomes systemically infected with *M.*

leprae. Bacteria in the liver and the spleen may reach levels of 10^{12} per g tissue. From such soft tissues it became possible to isolate *M. leprae* in quantities as great as might be obtained from bacteriological media (though with rather more trouble) [34], so that a serious study of the properties of the organism could begin.

In spite of the extraordinary levels of bacteria reached in the tissues of infected armadillos, there is no evidence that growth rates differ from those measured in the mouse. It is essential, as far as it is possible, to check that all the properties of the (abundant) armadillo-derived bacteria accord with those of the (meager) *M. leprae* obtained from man or mouse. Armadillos seem to be susceptibile to other mycobacterial infections—infections with cultivable mycobacteria [35, 36] and with a natural leprosy-like infection [37] have been noted in some animals. Since the animals cannot yet be bred in captivity, specimens for experimental use must be captured from the wild state. The possibility that a pre-existing mycobacterial infection may escape detection before the animals are experimentally infected with *M. leprae* should be remembered.

Other animal models

Two other animal models should be mentioned briefly: the nude mouse (congentially athymic) is highly susceptible to infection with *M. leprae* [38]. Such immunodeficient mice need a highly protected environment and the inconvenience of keeping them is probably not much different from that of keeping armadillos. They do, however, offer an alternative "production" source of bacteria. The grey mangabey monkey has recently been shown to be susceptible [39]. This model will probably be important for studies of pathology, since the disease seems to mimic human leprosy closely, but it is unlikely that a primate species would be a practical routine source of bacteria.

ULTRASTRUCTURE

Studies of the ultrastructure of *M. leprae*, in sections and as whole bacteria from man, mouse and armadillo, have been extensive but have not, for the most part, showed any unique feature of the organism compared with other mycobacteria. [For a general review of the ultrastructure of mycobacteria see [40].] A special problem with *M. leprae* is the difficulty of ensuring that the cells examined are viable ones, and that the ultrastructural features observed are not artifacts of degenerating bacteria. Although not a routine taxonomic technique, electron microscopy has been important in helping to establish the unrelatedness of *M. leprae* and *M. lepraemurium*. The latter seems to be a hard-to-cultivate strain of *M. avium* [31] and possesses a fibrillar capsule of polar glycopeptidolipid which is characteristic of species related to *M. avium* [42]. *M. leprae* never produces this kind of capsule [43, 44].

The wall

Four ultrastructural features may be characteristic of *M. leprae*: aberrant morphology, "wall bands," a symmetrical membrane, and "paracrystalline bodies."

Departures from the classical cylindrical shape of a bacillus are common in suspensions prepared from infected armadillo tissues (P. Draper, unpublished observations); typically cells have a tapered or double-tapered shape with hemispherical ends. One may infer some fault in the normal process of cell-wall construction, perhaps related to the intracellular environment. It is likely that the same aberration is seen in the light microscope as "club forms" [45].

Wall bands [46] are circumferential ridges on the outer surface of the cell (not to be confused with the randomly arranged "paired fibrous structures" common to all mycobacteria) [47]. Although the original publication records the presence of the bands on a strain of *M. avium*, the published pictures are not convincing and in this author's experience the bands are much less easy to see on other mycobacterial species than on *M. leprae*. They have been unmistakably observed on *M. lepraemurium* grown *in vivo* [44]. On *M. leprae* they can be very numerous and apparently positioned at random along the length of the cells. I have speculated [40] that they are scars left when the wall separates during the division process—such scars are known in other bacterial species [48] and (on a larger scale) in yeasts [49]. Their apparently random positioning (division scars should be near the poles of the cell) may again indicate a fault in the process of wall construction.

The membrane

The observation concerning the peculiar nature of the mycobacterial membrane of *M. leprae* is a recent one ([50]) and needs to be confirmed with a greater variety of infected tissues and suspensions of *M. leprae*, but it is of great interest. In general, bacterial membranes have the familiar triple-layered appearance in EM sections, with electron-dense outer layers and an electron-transparent inner layer. After carefully controlled fixation the images obtained of mycobacterial membranes may be measured with a densitometer to confirm the visual impression that the outer (wall-side) layer is thicker than the inmost electron-dense layer: the membrane, after preparation for sectioning, at least, is asymmetrical. In carefully fixed *M. leprae* in human biopsy specimens the membrane is, even in apparently intact bacteria, symmetrical. The cause of the asymmetry normally found is unknown, but presumably relates to an uneven distribution of membrane components able to react with the various electron-dense fixatives and stains used in the processing.

Paracrystalline structures

In sectioned *M. leprae* a quasi-crystalline body may sometimes be seen ([51]), consisting of an assembly of particles about 8 nm in diameter. This was originally thought to be associated with a bacteriophage infection but is now known to appear also in normal cells ([52]). The nature of the particles is not known and their relation to superficially similar particles which appear in *Escherichia coli* that has been stored at 4°C ([53]) is uncertain, but they have been observed in *M. leprae* alone among mycobacterial species.

CHEMICAL COMPOSITION

Wall chemistry

Further support for the mycobacterial nature of *M. leprae* comes from the comparison of its wall chemistry with that of other mycobacteria, since mycobacterial walls are chemically as well as ultrastructurally distinctive ([54]). They contain about 50% by weight of lipid in the form of high-molecular weight mycolic acids ([55]). These substances are of taxonomic interest because they differ between the closely related genera *Mycobacterium, Nocardia* and *Corynebacterium* as to molecular weight ([56]), and between individual species and groups of species of mycobacteria as to detailed chemical structure. Species or groups of species of mycobacteria exhibit characteristic patterns of mycolic acids which are easily observed by thin-layer chromatography ([57]). Mycolic acids from human- and armadillo-derived *M. leprae* are similar ([58–60]) and are of the high-molecular weight mycobacterial type but are in detail different from those of other species of mycobacteria ([61]). The latter point is reassuring in that it should be possible to distinguish between intentional infections of armadillos with *M. leprae* and natural infections with other species of mycobacteria. Mycolic acids of bacteria isolated from many leprosy-infected armadillos have now been studied in greater or lesser detail; they are apparently homogeneous. There is some debate about whether there are two or three species of mycolate present ([61–63]), but if a third component *is* present the amount is very small.

The rest of the chemistry of the wall seems to be similar to that of other mycobacteria except for one curious detail. All mycobacteria (and most other bacteria) contain a small peptide as part of their basic wall structure ([54]), the peptidoglycan. In the commonest variety of this peptide (shared by the mycobacteria) the sequence, starting from the amino-terminal end, is L-alanyl-D-isoglutaminyl-*meso*-diaminopimelyl-D-alanine. In *M. leprae* the L-alanine is replaced completely and specifically by glycine ([59, 64]). Such replacement is known in only one other species of bacterium ([65]) and in no other mycobacterium. It may thus be an important taxonomic marker, although the large amount of material needed to check it has confined information so far to armadillo-derived bacteria and must rule out its routine use.

Lipids

Mycobacteria characteristically produce a variety of unusual lipids that have long intrigued chemists. Two lipids produced by *M. leprae* are of special interest. On the one hand they show that this organism is capable of synthesizing typical complex mycobacterial lipids and on the other, that such lipids can be taxonomically useful in distinguishing *M. leprae* from other mycobac-

teria. Both the lipids seem to be extracellular; the bulk of them is found in the supernatants of homogenates of leprosy-infected tissue (human or armadillo) after the bacteria have been centrifuged down ([66–69]). However, their chemistry makes it clear that they are bacterial products.

The first lipid is a serologically active glycolipid ([70]), closely related to mycoside A produced by *M. kansasii* ([71]), but containing a unique trisaccharide which is presumably the antigenic determinant ([67]). Leprosy patients and infected armadillos form antibodies to this lipid ([72]). It is clear from the known distribution of such lipids among mycobacterial species that the leprosy glycolipid is likely to be antigenically as well as chemically unique. Production of related glycolipids is confined to a small group of species including *M. bovis* (but not *M. tuberculosis*) and *M. kansasii* ([71]).

The second lipid, which is structurally related to the glycolipid but contains no carbohydrate, is phthiocerol dimycocerosate (PDIM) ([68]). This substance too is confined to a small group of species ([71]). In general the PDIM of *M. leprae* is similar to those produced by other species, but details of the molecule seem to be unique ([69]). Thus, provided enough material can be accumulated, it should be possible to use this lipid to identify *M. leprae.*

BIOCHEMISTRY AND METABOLISM

Classically, the identification of bacteria has depended in part on so-called biochemical tests—the ability of an organism to oxidize or to hydrolyze various substances, for example. While there is a need to develop such tests for *M. leprae* for taxonomic purposes, there are several more basic reasons why an understanding of the metabolic processes of the organism are important. First, the effectiveness of drugs active against leprosy is already seriously compromised by the development of drug-resistant strains; understanding its metabolism may provide clues as to possible inhibitors that might be used as drugs. Secondly, the cultivation of the organism in a bacteriological medium is an important aim. The only alternative to random selection of possible ingredients of a suitable medium must be an understanding of the nutrient requirements derived from knowledge of metabolism. Finally, *M. leprae* is an intracellular pathogen. Consideration of the bacterial loads achieved in the armadillo indicates how successful it is at exploiting this environment. It is of both practical and "basic" interest to understand how the organism survives in its environment and what use it makes of the potential nutrients and metabolites available in the host cell.

The problem of purity

Biochemical or metabolic experiments with a pathogen that must be derived from tissues of an infected animal are complicated by the possibility that residues of the host tissue may themselves show metabolic activities. The rigor of possible purification methods is limited by the need to avoid damage to the pathogen itself. Thus each observed activity must be checked to ensure that it is a true activity of the pathogen. Examples of how such a distinction may be made are: 1) knowledge that a particular enzyme or pathway of metabolism does not occur in the host (most of the chemical structures described above are known to be products of *M. leprae* for similar reasons); 2) differences in physicochemical or kinetic properties between activities supposedly of the bacteria and those found in the host tissue; and 3) lack of effect of powerful reagents known to remove contaminating host proteins from the surface of the bacteria (but these reagents will probably also damage bacterial surface components). Many of the earlier reports of metabolic activities of suspensions of *M. leprae* isolated from human or animal sources are open to the criticism that no attempt was made to assess the amount of contamination by host material. Such contamination would be expected on the basis of work done with *M. tuberculosis* grown *in vivo* ([73]) and has, in fact, been confirmed in the case of *M. leprae* (see below). The present discussion is, as far as possible, confined to activities for which there is good reason to believe that *M. leprae,* not host tissue, is responsible.

DOPA oxidation

Probably the best-publicized biochemical activity of *M. leprae* is its ability to oxidize diphenols ([74]); D-dihydroxyphenylalanine

is a favored substrate because animal diphenoloxidases cannot oxidize this isomer ([75]). The activity seems to be unique to *M. leprae* among mycobacteria ([76]). Some fungi possess the enzyme but fungal contamination may be controlled. The activity, which has been detected in both human- and armadillo-derived bacteria ([77, 78]), may be measured by the formation of colored products ([77]), by the incorporation of radioactive DOPA into insoluble material in (or on) the bacteria ([79–82]) or by the release of radioactive products from labelled DOPA ([77]). Uptake of DOPA has been proposed as a rapid method of measuring bacterial viability and as a method of assessing resistance to antileprosy drugs.

It seems clear that the ability to oxidize DOPA is a true activity of *M. leprae* and may be used to identify the bacterium ([83–85]). Its significance is less clear. Evidence that the activity is an enzyme depends largely on its heat-lability ([86]); an alternative suggestion, that it is due to host components bound to surface structures of the bacterial cell, has been made ([87]). Such components might bind metal ions; the activity is inhibited by various metal-chelating agents ([75, 88]) but either an enzymic or a non-enzymic oxidase of this type might depend on metal ions. If the activity is indeed an enzyme it is not clear what its function is. Concentrations of DOPA in macrophages, the main host-cell for *M. leprae,* are unknown but unlikely to be high since these cells do not form melanin. It is now becoming clear that *M. leprae* is able to assimilate many compounds that *do* occur in macrophages and that lie on major normal pathways of bacterial metabolism (see below). It seems that DOPA oxidase activity must remain a useful anomaly until metabolic pathways in *M. leprae* are better understood.

Thymidine uptake

Suspensions of *M. leprae* are able to take up radioactive thymidine ([81]) (this activity may also be measured inside macrophages—see above). The uptake is small and very irregular. Although it has been proposed as a way of measuring bacterial viability and drug sensitivity, it is likely to be too small and too liable to error through (minor) contamination by other microorganisms to be routinely useful.

Oxygen metabolism and hydrolases

Work with highly purified suspensions or extracts prepared from such suspensions has recently allowed a fairly large range of activities of *M. leprae* (grown in armadillos) to be measured. These include some enzymes involved in dealing with oxygen metabolites ([89, 90]), which are believed to be among the microbicidal agents produced by macrophages. The superoxide dismutase is of the usual mycobacterial type. Curiously, catalase of mycobacterial origin seems to be absent, although host-derived catalase is present ([89, 91]), and it would be interesting to know how *M. leprae* protects itself against the potentially toxic hydrogen peroxide produced by macrophages.

There are a number of hydrolytic enzymes which seem to be at or close to the surface of the bacterium ([92]), where presumably they are well placed to hydrolyze substrates in the environment. Work on the hydrolytic enzymes has shown that host-derived (lysosomal) hydrolases are also present ([92]) tightly bound to the bacterial surface, as had been previously noted with *M. tuberculosis* grown *in vivo* ([73]).

Intermediary metabolism

It seems that *M. leprae* can take up glucose and amino acids ([93]). The latter are incorporated into trichloroacetic acid-insoluble materials while glucose is oxidized by both of the well-known routes, the Embden-Meyerhof-Parnas pathway (glycolysis) and the pentose-phosphate pathway ([94]). The working out of the main metabolic pathways of *M. leprae* is still far from complete but it is already clear that the organism contains much of the normal biochemical machinery used by microorganisms to maintain and reproduce themselves. There is no evidence so far for major deletions in metabolic pathways which might explain the dependence of *M. leprae* on host cells. In the case of *M. lepraemurium* it was inferred ([95]) that there were several "gaps" in metabolic pathways signalled by the need for growth factors in the medium.

Two interesting activities detected in, and apparently unique to, *M. leprae* are concerned with the metabolism of amino acids. At present the significance of neither reaction is clear. Glutamate decarboxylase

produces γ-amino-butyric acid (a neurotransmitter in animals) ([96]). γ-Glutamyl transferase can transfer glutamic acid residues to suitable peptide acceptors ([97]). An analogous activity occurs in mammalian tissues, where it is involved with metabolism of glutathione, but the enzyme in *M. leprae* may be distinguished by its ability to use D- as well as L-peptides or amino acids as acceptors. Both these activities might, in principle, be used to identify *M. leprae.*

Adenosine triphosphate pools

An important technique used in the refinement of the medium needed to support growth of *M. lepraemurium in vitro* was the measurement of ATP ([95, 98]). High levels of this nucleotide, which may be regarded as a sort of "energy currency" of the living cell, indicate active metabolism. Hence a medium that allows ATP content of bacteria to increase must be more nearly complete than one in which the level is static or declines. Very sensitive methods exist to measure ATP. It has already been shown that levels in *M. leprae* freshly isolated from animals are low but relatively stable ([99, 100]); whereas in *M. lepraemurium* the levels decline rapidly at first after isolation ([95]).

MYCOBACTERIOPHAGES

Mycobacteriophages have been much used to assist in the classification of mycobacteria, since different species or strains have different spectra of sensitivity. There is some evidence that *M. leprae* can at least bind the phage D29 ([51, 101]). Evidence for multiplication of the phage within the bacteria was equivocal, and a clear demonstration of this probably needs a medium able to support growth of *M. leprae.* Unfortunately, D29 is a phage of broad specificity, so that the result does not give any significant information either on the nature of the bacterial surface or on the taxonomic relations between *M. leprae* and other mycobacteria. Nevertheless, the results provide encouragement for further experimentation.

CULTIVATION *IN VITRO*

M. leprae, as defined at the beginning of this review, has not been cultured outside an animal cell ([102]). The reasons for this are not at present evident, but means at last exist, as indicated above, to understand how it may be grown *in vitro.* What, then, of the organisms cultured from leprosy tissue, some of which formerly appeared in culture collections as "*M. leprae*"? An obvious possibility is that they are contaminants; the skin is notoriously not sterile or sterilizable. This hypothesis is hard to test without extensive attempts to cultivate similar organisms from skin samples of normal people. Perhaps the simplest way to demonstrate that such organisms are contaminants is to show that they are not related to *M. leprae* or to leprosy chemically, biochemically, immunologically or pathologically.

The range of culturable organisms

One observation that might be taken to support a connection between the culturable organisms and leprosy is that the variety of strains isolated is restricted. Apart from the organisms isolated by Chatterjee ([103]), which are morphologically variable but mostly coccoid, they fall into two groups: acid-fast bacteria related to *M. scrofulaceum* ([104]) [including the Skinsnes organisms ([105, 106]) and the ICRC bacillus ([107])] and corynebacteria [LDC; isolated, for example, by Barksdale ([108]), Delville ([109]) and Reich ([110])]. When these strains were first isolated there was so little information on which to classify *M. leprae* that it was not possible to support experimentally the view that these organisms were radically different from *M. leprae*; the possiblity existed that they were *M. leprae* or at least that they were the etiological agent of leprosy and that Hansen's bacillus was some sort of nondividing derivative or side product. That view cannot now be maintained. *M. leprae* has mycobacterial complex lipids (see above) and immunological resemblances to other mycobacteria ([111]). It is recognizably different from *M. scrofulaceum.* It is capable of at least many major metabolic activities (see above) and dividing forms are commonly seen in tissues ([112]).

An ingenious solution to the problem of the gross differences between the organism isolated from tissues and the organisms cultivated in media is the concept of the "Janus Face" ([104]), i.e., a coordinated change in several microbial properties related to a large change in environment. Such massive changes are known, for example, in facul-

tative anaerobes where whole biochemical pathways can appear or disappear. Apart from the problem that *M. leprae* seems to be a three-headed Cerberus rather than a two-faced Janus, this hypothesis remains to be disproved.

DNA composition

Very recent results of the measurement of base compositions and homologies between *M. leprae* and various other organisms show surprising relations ([113]). None of the authentic mycobacterial DNAs was closely homologous to that of *M. leprae*; the closest were *M. scofulaceum* and *M. tuberculosis* (DNAs 58% and 52% homologous with *M. leprae*, respectively). This indicates clearly that *M. leprae* is not merely a strain of one of the species tested. DNA from one strain of LDC was 68% homologous with that of *M. leprae*. The G + C ratio of the *M. leprae* DNA was lower than the typical value for mycobacteria but similar to that found in corynebacteria. The length of the genome of *M. leprae* was much less than that of the cultivable mycobacterial species but similar to that of some of the LDC. Application of similar techniques to a large range of LDC ([114]) [which are typical corynebacteria ([115])] showed that there seemed to be two groups with high internal homology. Both groups seemed to be related to, but distinct from, human pathogenic corynebacteria. However, more recent unpublished work has yielded even more remarkable results (Dr. T. Imaeda, personal communication). Apart from confirming the genetic near-identity of human-derived *M. leprae* and the naturally occurring leprosy-like infection of armadillos, it seems that at least some strains of LDC have DNA showing a >95% homology with DNA from *M. leprae*. Such close similarity implies that the LDC may be as closely related to *M. leprae* as strains within a species.

LDC are clearly classifiable as corynebacteria related to human pathogenic species but distinct from them. They have typical corynebacterial properties, that is, they do not possess the special mycobacterial features found in *M. leprae*. Thus there seems to be a paradoxical discrepancy between genotype and phenotype. Clearly a confirmation of the DNA results and an extensive comparison between LDC and *M. leprae* are essential. The question of their respective pathogenicity seems never to have been tackled rigorously.

There may be a middle way between the extreme positions that *M. leprae* and the cultivable organisms from leprosy tissues are either unrelated or identical. That is, that the special immunopathological features of leprosy create an environment favorable for the culturable species. All the organisms are immunologically related (as are all mycobacteria, nocardias and corynebacteria) but a few types may have some special immunological feature or may possess other characters that allow them to grow in tissues if the immunological conditions are favorable. Now that supplies of host-derived *M. leprae* are sufficient to allow proper comparisons with the culturable species it is possible to test some of these very speculative notions.

PROSPECTS

Since the beginning of the second century of our acquaintance with *M. leprae* progress has been rapid. Knowledge of its main metabolic pathways should soon be complete and an understanding of how it survives inside the host cell should follow. Cultivation is an important aim, especially if the cultivated organisms share with those grown *in vivo* the important immunological properties needed for a vaccine. Even if this is not the case, cultivated strains will be invaluable in the development and testing of new drugs for use against leprosy.

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