the destruction of the perineurial sheath would seriously change the endoneurial environment, thus diminishing nerve conductivity and also allowing the entry of macrophages and lymphocytes.

6. In all the mouse models, striated muscle fibers were frequently seen to contain bacilli. In human leprosy although smooth muscle such as the arrector pili and dantor muscles are frequently infected, striated muscle is less so.

Preliminary histopathologic studies in susceptible armadillos also show the importance of hematogenous spread, including infection of nerves, nose with positive nasal discharges and most other sites common to man and mouse. In the armadillo the cellular picture at autopsy resembles L.L type leprosy. However, in the armadillo, atypical sites, including the lung, are heavily infected, possibly because of lower body temperatures.

Clinical implications. The significance of these models for studying clinical leprosy is that they reproduce, or can be adapted to reproduce, many of the features of leprosy in man. They particularly provide models for studying early phases of the evolution and pathogenesis of leprosy that can never be undertaken in man. These features are of particular importance for studying the pathogenesis of leprosy neuritis and possible routes of infection via the lungs, nose or gastrointestinal tract. Mice provide precise models for unravelling the immunological complexities of leprosy, bearing in mind that the majority of patients with leprosy are in the TT to BB range and these are the type of leprosy seen in normal mice. The armadillo, on the other hand, may in addition provide models for studying innate susceptibility and resistance and their possible genetic bases.

CONCLUSIONS

The report summarizes the considerable advances that have been made in experimental leprosy using animal models in a period of only 13 years. Thus, animal models are proving to be as valuable in leprosy as they have been for studying other human infections. The particular merits of the various animal models available for studying leprosy are discussed.

REFERENCES


COMMITTEE 2: ADVANCES IN THE MICROBIOLOGY OF M. LEPRAE

Members

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The report summarizes the progress made in the field of general microbiology of M. leprae during the past five year period since the Ninth International Leprosy Congress in London in 1968. M. lepraemurium has been included in the review as an interim model for M. leprae. Progress has been made in four areas: cytoology, metabolism, cultivation, and the identification of M. leprae.

CYTOLOGY OF M. LEPRAE

Morphologic Index (solid ratio). The utility of the Morphologic Index (MI), based on the proportion of solidly staining M. leprae cells has been exploited particularly to follow the initial antimycobacterial drug action in patients during chemotherapy. The present MI does not distinguish the infectious from the noninfectious patient. No unified opinion has been formulated regard-
ing the question whether the ratios of solid staining bacilli are associated with viability in the bacteriologic sense. Further studies appear necessary.

**Pyridine-extractable acid-fastness.** *M. lepra* in smear for smears lose the property of acid-fastness, but not gram-positivity when extracted with pyridine. The acid-fastness of *M. lepraemurium*, *M. tuberculosis*, BCG and *M. intracellulare*, is not affected by this procedure. It has been suggested that a differentiation between *M. lepra* and other mycobacteria is possible by using Ziehl-Neelsen staining after extraction with pyridine. Further studies are needed with *M. ulcerans* and *M. marinum* from human lesions, with *in vitro* grown mycobacteria and with senescent populations of cultivable mycobacteria.

**Electron microscopy.** Studies with the combined use of electron microscopy and chemical and biological techniques have been carried out. Miscellaneous information such as the band structure and peptidoglycolipid filaments on the surface of *M. lepra*, chemical components of the cell wall of *M. lepraemurium* and the electron-transparent capsule-like outer zone around the bacilli were obtained.

As a significant discovery, the mycolic acids in the cell wall of *M. lepraemurium* have been demonstrated to differ from corymycolic or nocardic acids. The characteristic mycolic acid-arabinogalactan-murain in the cell wall of *M. lepraemurium* resembles that in other members of the genus mycobacterium such as *M. tuberculosis* bovis and strain BCG. The discovery of mycolic acid in *M. lepra* isolated from human tissues likewise indicates that this pathogen is a mycobacterium.

In the field of electron microscopy, further chemical and biochemical information would permit the interpretation of the relationship between structure and function or the physiologic state of *M. lepra*.

**METABOLISM OF M. LEPRAE**

The extraordinarily long generation time of 12-14 days in the mouse foot pad has been regarded as one of the characteristics of *M. lepra*. No information is available to explain this slow rate of metabolism. One difficulty in metabolic studies on *M. lepra* is that of obtaining adequate supplies of cells and a second is that of collecting all the bacilli as suspensions without tissue contamination.

Fragmented reports have appeared indicating the presence of various enzymes in *M. lepra*. Among these, *o*-diphenoloxidase has been suggested to be unique to *M. lepra*, being distinct from plant and mammalian enzymes. Concentrates of *M. lepra* prepared from lepromatous material actively oxidized, 3,4-dihydroxyphenylalanine (DOPA) to pigmented products. This specific metabolic activity has been proposed as an identification test for *M. lepra*.

Ribulose diphosphate carboxylase activity was demonstrated in the supernatant from disrupted *M. lepra* collected from lepromatous tissues. It appears to be important if confirmed, because this enzyme occurs otherwise only in autotrophic bacteria and green plants.

Recent evidence has suggested an incorporation of tritiated thymidine into leprosy bacilli in cultures of human lepromatous macrophages. If the observation is confirmed, this is an important advance, because it implies that the organisms were synthesizing DNA. Further studies of this type are needed.

**Metabolism of M. lepraemurium.** Since the last congress, knowledge of the metabolism of *M. lepraemurium* increased dramatically. The major advance is in our knowledge that the overall energetics of the organism operate independently from those of the host. It has been demonstrated that *M. lepraemurium* contains a cytochrome-linked pathway for oxygen utilization. Due to the limited rate of terminal electron transfer, the assimilation and oxidation of exogenous substrates occurs very slowly and does not result in marked stimulation of oxygen uptake.

Experiments using isotope-labeled substrates and cell-free extracts of *M. lepraemurium* have confirmed the following facts:

1. An extraordinarily slow rate of aerobic metabolism based upon a host-independent tricarboxylic acid (TCA) cycle. All enzymes of the TCA cycle have been demonstrated, the pyruvate and *α*-ketoglutarate dehy-
drogenases being rate limiting. Although the TCA cycle may contain an alternative pathway at the \( \alpha \)-keto­glutarate step, a conventional TCA pattern of isotopic distribution arose during substrate oxidation.

2. There appears to be a lack of capacity to oxidize glucose, even though its incorporation into cellular material has been established.

3. Short-chain fatty acids, in contrast to medium-chain fatty acids, cannot be utilized for lipid synthesis.

Studies on energetics have been further advanced by the development of ultrasensitive methods for determining ATP (energy levels) in host-grown microbes. Host ATP has been eliminated and the quantitation of ATP refined to require only \( \frac{1}{60} \)th the number of bacterial cells employed in the most sensitive methods hitherto available. The method has been applied thus far to demonstrate the potential losses of ATP during extraction, purification, prolonged refrigeration, and the growth potential of \( M. leprae \) in cultivation studies. These methods have been designed to investigate the energetics of \( M. leprae \).

CULTIVATION PROBLEM

Cultivation of \( M. leprae \). Four cell-free systems have been described for the cultivation of \( M. leprae \) in vitro. These are: a) a U-tube divided by a fine sintered glass membrane and using a conventional medium for mycobacteria, b) an inorganic medium suitable for autotrophic bacteria, c) semi-soft agar media, and d) media enriched with substance of mycobacterial origin. These experiments have not been successfully repeated by other investigators.

Attempts at cultivation in cell cultures were carried out using cell lines of human origin, cell strains derived from human tissues, cell strains derived from animals, and mouse macrophages. No proliferation of the bacteria was noted.

Recently, evidence has been obtained regarding a limited multiplication of \( M. leprae \) within macrophages derived from human peripheral blood cells. The applicability of the method is restricted by limited survival of the host cells. The majority of host cells did not survive beyond 60-80 days in most cultures.

Cultivation of \( M. leprae \) in vivo is problematic. The growth medium has not been found to support the growth of \( M. leprae \) in vitro. The WHO Expert Committee on Leprosy 1970 presented a report on the identification of \( M. leprae \) as follows:

"In all the cultivation work, it is important to prove the viability of the purported growth and to identify it by the methods now available (the inoculation of mouse foot pad, lepromin test, enzymatic studies of \( \text{DOPA} \) oxidation, and serological identification of nodular extract antigens)."

The pyridine sensitivity and the specific \( \alpha \)-phenoloxidase in \( M. leprae \) have already been described.

It has been observed that leprosy nodular extract (NE) contained at least two antigens which have been differentiated from human serum proteins by the immunodiffu-
tion test. One of these antigens is a heat-
stable polysaccharide, and the other is a
heat-labile protein which give a single pre-
cipitation line with anti-NE serum ab-
sorbed with human serum. Inasmuch as
this antigen is highly specific for M. leprae,
serological identification of M. leprae by
the technic of immunofluorescence should
become practicable with antiserum pre-
pared against the antigen.

COMMITTEE 3: ADVANCES IN EXPERIMENTAL CHEMOTHERAPY

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Progress in the last decade now allows
the same topics to be studied in leprosy as
in other bacterial diseases. They are:

1. Screening of new drugs, and determi-
nation of minimal effective dosage
(MED).
2. Characterization of antileprosy drug
activity: bactericidal, bacteriostatic,
or bacteriopausal (prolonged bac-
teriosis).
3. Methods of measurement of drug in
blood and tissue, determination of
minimal inhibitory concentration
(MIC), and pharmacokinetics includ-
ing repository effect.
4. Drug toxicity in relation to MIC.
5. Metabolism of the drug.
6. Short-term clinical trials to determine
whether a given drug is also active in
man.
7. Long-term clinical trials to determine
whether a drug's activity continues to
the point of smear-negativity.
8. Very long-term follow-up to see if
smear-negativity is maintained or if
drug-resistant M. leprae eventually
emerge.

These steps should be followed in the
development of antileprosy drugs. Patients
should not be deprived of standard dapsonen
(DDS) therapy in order to test compounds
that have not been tested against M. leprae
in animals or compounds that appear on the
basis of results in animals to be clearly less
efficacious than standard therapy.

DRUG SCREENING AND
CHARACTERIZATION OF
ANTILEPROSY ACTION

Experimental model. In the absence of
significant growth of M. leprae in vitro, all
work must be done in animals. Most re-
search has been done in the mouse model.
This infection is very consistent. Geneti-
cally uniform mice are readily available and
easily maintained in standard conditions.
Hence, the mouse continues to be the ani-
mal of choice. Other animals may be useful
when particular findings must be checked
in another species. For studies requiring
larger populations of M. leprae, the thym-
mectomized-irradiated mouse, the neonatal-
thy-mectomized rat and the armadillo
may provide suitable animal models.

Methods of study. The continuous method
of drug administration (from the day of in-
fec tion to the end of the experiment) re-
veals whether a drug is active against M.
leprae. The kinetic method (administration
of drug during a limited period, beginning
early in the logarithmic phase of growth of
bacilli), determines whether a drug pro-
duces bactericidal, bacteriostatic or bac-
teriopausal effects. Administration of drugs
in graded dosages allows the MED and
MIC to be determined.

Results of drug screening and characteri-
ization. With these methods, more than 200
drugs have been tested. Only a few have
exhibited bactericidal (or bacteriopausal)