Volume 48, Number 4
Printed in the U.S.A.

BOOK REVIEWS

Mutatkar, R. K. *Society and Leprosy*. Pune, India: Sharad Gogate, 1979, 297 pp. Price: Rs. 30.00.

Health education in leprosy is, as Mark Twain remarked of the weather, a subject that receives a good deal of talk but little action. For example, a 5 year (1972–1976) review of three leading leprosy journals revealed only three articles directly relating to leprosy health education. This situation, of course, was reflective of the attitude toward health education in general; for example, in 1973, it was reported that only one-half of one percent of the U.S. health dollar was allotted for health education and that the U.S. Department of Health, Education and Welfare estimated that it spent no more than one-fifth of one percent of its budget on health education.

Society and Leprosy is a 297 page hardbound publication that attempts to draw attention to the need for a more rational approach to health education by the leprosy community. More specifically, it is an objective assessment of the health education program of the Gandhi Memorial Leprosy Foundation, conducted by the Department of Anthropology, University of Poona. The study itself consisted of a comparative analysis of two randomly selected stratified population samples from Poona (experimental) and Jalgoan (control) with regard to knowledge levels and attitudes toward leprosy. Ten case studies are also presented for qualitative analysis purposes. Generally, results of the study confirm the effectiveness of the health education program at Poona, particularly with regard to an increase in knowledge levels and resultant attitudinal change. As is perhaps to be expected, prejudices rooted in deep cultural patterns are difficult to affect through education.

Hopefully, this study will serve as a harbinger of things to come in future leprosy health education programs. Evaluation studies in health education admittedly are difficult, both in design and interpretation. However, *Society and Leprosy* shows that it can be done, and, in so doing, we at last will be in a position to more realistically assess the actual value of health education in leprosy control programing. Accordingly, a great debt of gratitude is owed to the Gandhi Memorial Leprosy Foundation for providing leadership in this regard.—R. J. O'Connor

Sehgal, V. N. *Clinical Leprosy*. Sahibadad, Distt. Ghaziabad, U. P.: 1979, 100 pp. Price: Rs 22.

Professor's Sehgal's book is a concise practical guide to the understanding of leprosy. As stated in the Preface:

"Leprosy is one of the major public health problems in developing countries. Its teaching should now form an essential content of the undergraduate curriculum in medical institutions in these countries. The clearcut understanding of clinical manifestations of leprosy is, therefore, imperative. The text of this book deals with different clinical facets of the disease, which are essential for the undergraduate and also postgraduate students. They are based on didactic lectures and clinical demonstration on leprosy, which the author has been imparting to students for the past two decades. The contents are carefully cast to meet the requirement of curriculum of most institutions. Emphasis has, however, been on the classical clinical features of different types of leprosy. The essentials of diagnosis and also the treatment have been clearly outlined. The recent concepts of pathogenesis of leprosy, mechanism of reactions, newer drugs, and dapsone resistance along with the status of leprosy control have been described. The latter, in particular, in the Indian sub-continent has been discussed. The generic as well as the trade names of commonly used drugs in leprosy is a unique feature of the text. The book is concise and comprehensive. It is well-illustrated with neat photographs. It is primarily written to cater to the need of students; it is hoped, however, that it may be useful also to practicing physicians and leprosy field workers in developing countries in particular."

The book provides valuable instruction to non-Indian leprologists in the diagnosis and management of the disease of that subcontinent. The BI, for instance, is explained on the 0–4+ scale in general use before Ridley's 0–6+ scale was introduced. Fluorescent microscopy is recommended to demonstrate bacilli when they are few in number or when the workload is very high in the laboratory. Epidemiological considerations focus on the experience in India. The pathogenesis and development of clinical types of leprosy are masterfully explained. Of particular value to an "outsider" are the clear explanations and comparisons among the Madrid (1953) Indian Leprologists' Association (1955), field worker's, and Ridley-Jopling (1966) classifications. Entire chapters are devoted to tuberculoid, maculoanesthetic, lepromatous, borderline (dimorphous), indeterminate, and polyneuritic leprosy. Reactions are considered as being either ENL or lepra reactions, the latter including reversal reactions and downgrading reactions. The chapter on therapy advocates the older practice of initial small doses of dapsone with gradual increments in the first 4–6 months and takes the position that dapsone must be used cautiously to avoid precipitating reactions. The illustrations are quite good. All in all, Professor Sehgal has admirably achieved his goal of providing a concise educational tool for medical undergraduates in developing countries.—RCH

The in vitro *Cultivation of the Pathogens of Tropical Diseases*. Proceedings of the Workshop held in Nairobi, Kenya, 4–9 February 1979. Basel: Schwabe & Co. AG, 1980, 472 pp.

The International Laboratory for Research on Animal Diseases (ILRAD) and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases jointly convened a workshop on 4–9 February 1979 in Nairobi, Kenya to review present knowledge in this field and to consider how research might be advanced. Organisms carrying the following diseases were considered: malaria, schistosomiasis, filariasis, trypanosomiasis, leprosy, leishmaniasis of man, and theileriasis in cattle. This book is an account of the workshop, which was held at ILRAD in Nairobi, Kenya.

The workshop considered working papers on the present state of knowledge and short communications on recent advances in pathogen cultivation. The working papers have been reproduced in full and the short papers as brief communications. The discussions which followed the presentations have been summarized by rapporteurs. The views here expressed are, of course, those of individual authors and do not necessarily reflect the policies of the Special Programme or of ILRAD. The rapporteurs drew on the discussions of the workshop in preparing the appendices.—D. S. Rowe, and H. Hirumi.

Session VI is entitled "Cultivation of leprosy pathogens" and contains the following papers:

Nakamura, N. Cultivation trials of *Mycobacterium leprae* in cell-free liquid medium based on culture methods for *Mycobacterium lepraemurium*.

In 1972, the author established a cell-free liquid medium, NC-5 medium, for the growth of *Mycobacterium lepraemurium*, which had previously been thought to be an obligate intracellular parasite. When *M. lepraemurium* was inoculated into NC-5 medium and incubated at 30°C, the number of bacilli increased up to the sixth week of cultivation. These results were confirmed by Dhople and Hanks in 1974 by means of ultrasensitive determination of ATP and microscopic measurement of total biomass. Dhople and Hanks have also improved the original culture medium, which is somewhat labile, and defined a stable medium in which continuous multiplication of *M. lepraemurium* is able to take place.

The process of establishing the NC-5 medium was as follows:

a) Discovery of a physiological condition for elongation of *M. lepraemurium* cells. A definite relationship was found between the elongation of cells and the depth of the Kirchner medium, which is routinely used for cultivable mycobacteria.

b) Establishment of an elongation medium (NK medium) for elongation of *M. lepraemurium*. It was found that α-ketoglutarate and oxaloacetate remarkably stimulated the elongation phenomenon.

c) Establishment of the NC-5 medium for quantitative multiplication of *M. lepraemurium*. This was accomplished by adding compounds such as cytochrome c, haemin, and l-cysteine (related to the respiratory and hydrogen transfer systems) to the NK elongation medium.

Since 1974, the author has been trying to improve the original NC-5 medium to one which might be applied to the experimental cultivation of *M. leprae*. The attempts to obtain a medium for culturing *M. leprae* were as follows:

a) Use of the Dubos medium as the basal medium (ND-base) instead of Kirchner medium because the Dubos base is more stable and more efficient than the Kirchner base.

b) Substitution of thioglycolate for l-cysteine, according to the suggestion made by Dhople and Hanks.

c) Addition of succinamide to the Dubos base because succinamide remarkably stimulates the growth of *M. lepraemurium*.

d) Addition of tyrosine and vitamin C to ND-base. Prabhakaran, *et al.* had determined that tyrosine is a substrate in the course of DOPA oxidation by *M. leprae*.

The author's experience with *Mlm* suggested that the ND-15 oxaloacetate medium containing tyrosine and vitamin C offered the best possibility for the multiplication of *M. leprae*.

The evidence presented here was obtained in cultivation experiments with *M. lepraemurium* and *M. leprae* and might contribute toward cultivation trials with other so-called "obligate intracellular parasites."—Author's Summary

Hanks, J. H., Dhople, A. M. and Funk, H. B. Fundamental problems of cultivating *Mycobacterium lepraemurium* and *Mycobacterium leprae* in cell-free systems.

Two species of mycobacteria have been regarded as "obligate intracellular microbes" because scores of investigators have been unable to induce their growth under bacteriologic conditions. The work on *Mycobacterium leprae* has been continuous since shortly after its discovery by Hansen in 1873. Nevertheless, this agent has not yet been grown in cell cultures or in bacteriologic media. *Mlm* (*Mycobacterium lepraemurium*) agent of rat leprosy has been the experimental model for *M. leprae*. This species was noncultivable from its discovery by Stefansky in 1903 until the convincing report of Nakamura in 1972, a period of 69 years. It has been grown in cell cultures since 1958.

The original Nakamura system consisted of a semisynthetic base for tubercle bacilli and seven supplements (in order of importance for *in vitro*-adapted type II cells): alpha-ketoglutarate, haemin, cystein; cytochrome c, goat serum; pyruvate, and calcium pantothenate. The unique feature of this system was that the cystein-containing medium occupied 65% of culture tube volume.

All cultivable intraphagosomal microbes exist in two differentiated states. Bacterial cells liberated from lesions are designated type I cells. They are deficient in cytochromes and have leaky cell membranes. Type II cells possess non-leaky, *in vitro*-type, cell membranes and are growth competent. It follows that the first hurdle in cultivating such agents is to maintain the metabolic integrity to type I cells while they differentiated to type II cells.

In the optimized Nakamura semisynthetic system, maximal growth rates occurred at 30°C, i.e., 8°C below host temperature. Growth potential was lost between the fourth and the sixth weeks. Twenty times the usual concentration of Mg ions was required for normal cell division. During serial transfers at 4 week intervals, the growth potential declined progressively (24× to only 9× in six transfers).

Continuous, transferable growth at 38°C requires: a) replacement of the most active supplements with stable compounds and b) unknown factors in yeast extract and tryptic digest of casein. The growth rates are 15× those in highly susceptible mice.

In our investigations ultrasensitive bioluminescence determinations of ATP have been employed to determine the physiologic status of frozen and refrigerated cells, to quantitate the merits of physicochemical conditions and compounds in the absence of growth, and also to quantitate cell crops during growth.—Authors' Summary

Pattyn, S. R. and Portaels, F. *In vitro* cultivation of *Mycobacterium lepraemurium*—Preliminary results.

Recently we took up again the *in vitro* cultivation of *Mlm* (*Mycobacterium lepraemurium*) as an introduction to the *in vitro* cultivation of *M. leprae*. As media we decided to test the Nakamura-Hanks liquid system in a couple of its multiple variants and the Ogawa egg yolk medium with which Ogawa and Mori have described some success.

Bacillary suspensions were obtained from spleen, liver, muscle, and bone marrow of intravenously (i.v.) inoculated mice. More than 5 years ago, Brown showed that *Mlm* in mice has a shorter generation time in bone marrow than in other organs and suggested that bone marrow might be the major site of multiplication in i.v. inoculated mice. Since early results were obtained with solid and not liquid media, and because of lack of facilities, further experiments were conducted with the Ogawa egg yolk medium and some simple variants: Ogawa with mycobactin from *M. phlei*, Ogawa with glutamine, and Ogawa with glycyl-histidine-lysine tripeptide (GHL) as used for the cultivation of mycoplasmata. The tubes were closed with screw caps, rubber stoppers, rubber stoppers transpierced with an injection needle, and stoppers with a cotton thread.

Colonies were obtained on the Ogawa-medium whatever the type of stopper, with or without glycyl-histidine-lysine tripeptide, without glutamine, and with or without mycobactin. Microcolonies appeared after 2 weeks and increased in size and number during the next 3 months. The number of colonies usually varied between two and 30. In six cases confluent growth was observed at the bottom of the slants, with or without condensation fluid. With our carbol-fuchsin stain, *Mlm* from infected tissue is always in a beaded form. In culture they presented themselves either as globi or as pleomorphic acid-fast rods with some branching and also with swollen ends. The latter are generally considered as a degenerative phenomenon. Therefore, ultrathin sections were prepared and examined by electron microscopy. In a young colony appearing within 1 month, healthy looking mycobacteria were seen, with occasional forms which had just divided and also very long forms. Some of these were filled with particles, which we could not identify. The particles were too large to be ribosomes and too small to be bacteriophages. Furthermore, such particles could be observed extracellularly but were clearly of intrabacterial origin. Both empty bacilli and broken bacilli liberating such particles were observed.

Colonies were observed in nine of 11 trials with *Mlm* and in five of six trials with a strain of *Mlm* derived from cat leprosy. Confluent growth occurred in six of the 200 tubes inoculated. Attempts to subculture have been frustrating. Whether transfers were made from young colonies or older colonies, the results have been erratic because on each occasion only one to three colonies grew. This is unusual in mycobacteriology, especially when subcultures are made from confluent growth. Media of different composition are now under investigation.—(*Adapted from* authors' summary)