Abstracts

1. MICROBIOLOGY


Natural infections with bacilli similar to *Mycobacterium leprae* have been found in 14 armadillos trapped in southern Louisiana in 1974 and 1975. Four of the animals had ulcers or subcutaneous nodules suggestive of leprosy. A nasal smear from 1 showed acid-fast bacilli. In 7 of the animals post-mortem examination has revealed invasion of dermal nerves, typical of leprosy. Examination of the other animals has not been completed. Attempts to culture the bacilli have been unsuccessful. Positive Mitsuda reactions at 28 days have been obtained in patients with leprosy tested with lepromin prepared from the tissues of these animals. The 14 infected armadillos represent about 10% of un inoculated animals examined from the trapping areas; most were examined within a month of capture. “The pathologic picture and the Mitsuda reactions at 28 days strongly suggest that the mycobacteria found in these wild armadillos are *M. leprae*, but until other pending confirmatory tests are available, the identification cannot be considered definite.” The original report on this finding is by WALSH et al., *J. Reticuloendothelial Soc.*, 1975, v. 18, 347.


“*Mycobacterium leprae* multiplied in media enriched with substances originating from other mycobacteria, from non-acid-fast *Actinomycetales* or from gram-positive or gram-negative *Eubacteriales*. Most of the *M. leprae* strains did not grow on a synthetic medium containing the amino acids present in *M. smegmatis*, but the growth-promoting effect of sonic extracts of this organism indicated that substances of bacterial origin, other than amino acids, do act with *M. leprae* was verified by their ability to oxidize D-3,4-dihydroxyphenylalanine (D-dopa test). *M. leprae* did not multiply on Nakamura medium unless at least 40% of medium NM7 enriched by bacterial substances was present. Adequate aeration was essential for multiplication of *M. leprae* in enriched NM7 and No. 3 media.”


The English summaries appended to the papers are as follows:—

1. "It was demonstrated that *M. lepraemurium* which were cultivated for 223 days at 30° C by bacillary suspended method and for 172 days by slide culture method, had the ability to infect susceptible mice. In both cases, the procedure of refreshing culture medium resulted in stimulation of the growth of bacilli. In the case of slide culture method, it could be noted that
the infectious abilities of the cultivated bacilli depended upon the number of living organisms, because the growth of bacilli was stimulated by a slide transfer procedure. On the other hand, no pathogenicity was demonstrated when the bacilli were cultivated for 64 days as the control in the EKP medium under the same condition.

"However, the quantitative observation about a relationship between the growth rate of bacilli and the grade of pathogenicity illustrated that multiplied bacilli had the reduced pathogenicities comparing to that of starting material."

ii. "Stabilities of the NC-5 and NC-7 medium for the growth of \textit{M. lepraemurium} were studied. For this purpose, these media were kept at 37°C and 4°C for 1 and 2 months. Using slide culture method, \textit{M. lepraemurium} were cultivated in a freshly prepared medium and the preserved media. In order to see the stabilities of the preserved media, the multiplication of \textit{M. lepraemurium} in the preserved medium was compared to that in a freshly prepared one. The results obtained show that the bacilli multiplied equally in both freshly prepared and preserved media in the case of NC-5 medium, and that, in the NC-7 medium the dominant growth was observed in the medium which was kept for 1 month at 37°C, and the inferior growth was seen in the medium preserved for 2 months at 37°C. Therefore, it could be concluded that the NC-5 medium would be very stable for use."

iii. The experiments of the factors influencing the growth of \textit{M. lepraemurium} smeared on slides and cultivated in NC-5 medium were carried out as following:

"1. Slide-transfer group: the group in which a smeared slide was transferred to a freshly prepared medium at a definite interval.

"2. Air-exposure group: the group in which a slide was just taken out and put again in the same medium at a definite interval.

"3. Stopper-opening group: the group in which a rubber stopper was just taken out and sealed again at a definite interval.

"4. Control: no treatment group.

"The growths of bacilli treated with the four procedures were compared. The most remarkable growth was observed in the case of the air-exposure group, and the inferior growth was obtained in control group. From the results, it could be presumed that periodical exchange of air in sealed culture medium might be necessary for the growth of bacilli.

"In the experiments of bacillary cultivation, the suspensions of bacilli were inoculated and cultivated in 50 ml of NC-5 medium, which were distributed to a 50 ml-flask 100 ml-flask, and 200 ml-flask, respectively. After two months' cultivation at 30°C, bacterial cells were collected by centrifugation and the wet weights of sediments were measured. The best yield of bacterial cells was obtained when the bacilli were cultivated in a 50 ml-flask, and the poor yield was observed in the case of a 200 ml-flask. Therefore, it would be presumed that \textit{M. lepraemurium} might multiply under a slightly anaerobic condition, rather than aerobic one.

"From the results of two experiments mentioned above, it could be emphasized that the growth of \textit{M. lepraemurium} would unexpectedly depend upon the influence of air."


"To define the minimal inhibitory concentration (MIC) of dapsone (DDS) for \textit{Mycobacterium leprae} in rats, we determined the relationship between dietary and plasma levels of DDS in uninfected male and female Lewis rats. This knowledge was applied to the design of experiments using rats inoculated in the footpads with \textit{M. leprae}. The MIC for DDS in male and female rats, respectively, was 1.5 to 4.0 ng and 1.8 to 3.0 ng of DDS/ml of plasma, even though the sexes exhibited markedly different concentrations of DDS when receiving the same dietary level of DDS. These values for the MIC of DDS for \textit{M. leprae} in rats are nearly identical to the previously determined MIC of DDS for \textit{M. leprae} in mice."

"Morphological features of *Mycobacterium lepraemurium* cultivated on a slide glass in a cell-free liquid medium, NC-5, were precisely observed by a scanning electron microscope. As previously demonstrated, elongation of bacterial cell was noted in the second weeks of cultivation, and thereafter septum formation, division, budding and branching followed. It was obvious that the growth grade of bacilli depended upon the number of bacilli presented on the slide glass; when a small number of bacilli was inoculated on the slide glass, a micro-colony was formed in 10 weeks' cultivation, but further development did not occur even if cultivation was continued."


A technically simple method is described for the separation of *Mycobacterium lepraemurium* or *M. leprae* with a degree of purity sufficient for use in spectrophotometric studies. The recovery rate for bacilli is high. The original paper should be consulted for a description of the method.

_D. S. Ridley_


This paper describes details of the method by which 1.6 mg of purified *Mycobacterium leprae* protein was extracted from 62 g liver of a heavily infected armadillo. This protein was used as a skin test antigen in groups of 5 armadillos either not vaccinated or vaccinated 8 months previously with heat-killed armadillo tissue-derived leprosy bacilli in Freund’s incomplete adjuvant. [The last paragraph on p. 145 is muddled with regard to controls—perhaps a sentence has been omitted?] Previously vaccinated armadillos developed erythematous areas 4-8 mm in diameter 24 hours after skin testing. These were fading by 48 hours. Skin test reactions elicited 12 months after vaccination persisted up to 72 hours. These reactions were taken to indicate delayed hypersensitivity to *M. leprae* protein. [The presence or absence of immediate (10 minutes) and Arthus (4 hour) sensitivity is not commented upon, nor the histology of the skin test response. The specificity of the response is not described although alluded to in the discussion.]

_A. D. M. Bryceson_

**2. IMMUNOLOGY AND PATHOLOGY**


This well illustrated account of the histological classification of leprosy is a record of the system used by the author which has been found to be applicable to all ethnic groups studied to date, as well as to experimental leprosy in mice, and includes a number of new concepts resulting from a re-evaluation of immunological criteria.

[This valuable paper does not lend itself to abstraction and should be studied in the original.]  

_W. H. Jopling_
Sub-epithelial humps in the glomeruli were seen on electron microscopy in a patient with lepromatous leprosy and erythema nodosum leprosum (ENL). The humps were of the sort known to occur in immune complex nephritis, and, since there was no evidence of recent streptococcal infection, the assumption is that the renal condition was due to ENL. The patient had microscopic haematuria and only trace proteinuria. The total complement and C3 levels were normal, possibly due to low complement consumption.

D. S. Ridley


"One hundred and forty-eight members from 30 families (64 children) from Ethiopia, where one or more persons were affected with leprosy, were investigated for genetic polymorphism of C3, serum concentration of β1C/β1A-globulin and immunoglobulins A, G and M using high voltage agarose electrophoresis, immunoelectrosay and single radial immunodiffusion techniques respectively. The results are compared with related healthy controls. No association between C3 phenotypes and leprosy could be established through family studies. C3 concentration was, however, lower in leprosy patients. Difficulties and drawbacks of such studies with small families are discussed."


"Occurrence of cold lymphocytotoxins has been observed in 59 leprosy sera. In 46% of the patients, cold lymphocytotoxins were present whereas in healthy controls only 13% showed such antibodies. The highest incidence of alloantibodies was detected in lepromatous leprosy. Levels of autoantibodies, immunoglobulins and C3 were tested in parallel without finding any significant correlations."


The English summary appended to the paper is as follows:—

"The immunological competence of 11 patients with indeterminate leprosy was compared with that of 10 normal volunteers of the same age and sex distribution: these controls have not had previous contact with leprosy.

"The following parameters were studied in peripheral blood cells: 1) percentage of lymphocyte bearing surface immunoglobulins, as revealed by immunofluorescence; 2) percentage of lymphocyte bearing complement receptors, as studied by antibody and complement coated erythrocyte rosetting; 3) percentage of T cells, as revealed by spontaneous sheep erythrocyte rosetting; 4) blastogenic and myogenic reaction of cultured lymphocytes to PHA, and 5) cell migration inhibition test using lepromin (80 x 10⁶ bacilli/ml) as antigen. Skin reactions to lepromin were also assayed. In the 6 lepromin-positive patients with indeterminate leprosy, no major immunological alterations could be detected. On the contrary, the 5 lepromin-negative patients showed important alterations which could well be considered as precursors of lepromatous leprosy."

A comparative evaluation of bacteriological and morphological indices (BI and MI) for leprosy bacilli was made in biopsies of skin, lymph node, sural nerve and quadriceps muscle and in marrow aspirates. There were 15 patients with untreated bacillary positive leprosy, including 5 with erythema nodosum leprosum (ENL) and 2 with dimorphous reactions. Although there were individual variations, neither BI nor MI was generally lower in skin than in any of the other tissues, with the important exception of the patients in reaction. In these patients the MI was often much higher in lymph nodes than in skin, and in a few patients this was also true of the BI. The BI of nerve and muscle was generally lower than in skin, except in one case of ENL and another of dimorphous reaction. The MI of muscle was zero in all biopsies.

[This paper brings out well the point that one reason for the finding of viable bacilli at sites other than skin might be that bacilli in skin (and nerve) are more subject to reactions.]

D. S. Ridley


Motor and sensory nerve conduction studies were performed on 93 Aborigines from the Northern Territory of Australia; 30 were control subjects, 36 were leprosy patients, and 27 had no abnormalities apart from one or more clinically enlarged nerves of unknown aetiology. In the leprosy group impairment of conduction was demonstrated in the vast majority of clinically abnormal nerves and also in many nerves which were considered normal on clinical examination. Furthermore, it was found possible to locate the segments of nerves in which damage was maximal. In the third group of subjects, abnormal conduction was demonstrated in nearly 50% of the clinically enlarged nerves, and later it was established that leprosy was the cause of the conduction defect in the majority of cases.

The authors conclude that observations on nerve conduction are of considerable value in the diagnosis and management of leprosy.

W. H. Jopling


"Biopsies from radial cutaneous nerves of 4 untreated lepromatous patients were studied using the electronmicroscope. It was found that M. leprae engulfed by Schwann cells grew, and multiplied, building up protective responses against the destructive action of the cell by losing their phagosomal membrane and by producing an inert electron transparent substance around them. However, once the organisms were dead they were digested inside phagolysosomes. The electron transparent substance produced by the cell-bacilli interaction might remain inside the cell for a long time giving it a foamy appearance."


"Study of the number of thymus-derived lymphocytes by the rosette assay (T-RFC) in patients with leprosy reveals that lower than normal numbers of T-RFC are regularly seen in those patients with the active lepromatous form of this disease. Essentially normal numbers of T-RFC were found in inactive lepromatous, borderline, and indeterminate types of leprosy. The lowest percentages and lowest absolute numbers of T-RFC were encountered in patients with
lepromatous leprosy resistant to chemotherapy. Patients with lepromatous leprosy complicated by erythema nodosum leprosum show numbers of T-RFC that are more nearly normal than the numbers of T-RFC in patients with uncomplicated lepromatous leprosy. These findings are discussed with respect to the pathogenesis of lepromatous leprosy and the T-RFC deficiency demonstrated in this disease. The possibility that transient defects in T-RFC numbers or function may predispose to lepromatous leprosy is proposed.”


“Phytohaemagglutinin-M induced lymphoblast transformation of peripheral lymphocytes in cell cultures was studied in 21 lepromatous and 7 tuberculoid leprosy patients and in 9 control subjects. There was no appreciable difference in the range or mean of the proportion of blasts in the cultures among the 3 groups. This indicates that thymus dependent lymphocytes are basically normal in number and function in lepromatous leprosy in our locality.”


“This investigation studied the possibility of activating lepromatous macrophages by a local ‘in vivo’ test.

‘Lepromatous macrophages have an evident incapacity for clearing M. leprae. This is demonstrated by injecting lepromatous patients with an antigen containing M. leprae from human tissue at a concentration of 640 × 10⁶ bacteria per ml. This produces a nodule which, at 30-day biopsy, shows a macrophagic granuloma with numerous bacteria inside the macrophages, proving that these cells are unable to remove M. leprae. This incapacity is specific for M. leprae, and all other mycobacteria produce a different reaction.

‘Local ‘in vivo’ stimulation of the lepromatous macrophage was obtained by injecting M. leprae in the same concentration as above but mixed with other mycobacteria (M. lepraemurium or BCG).

“The mixed antigens produced a tuberculoid granuloma with abundant lymphoid cells. Fite–Faraco stains showed almost no acid-fast bacteria. Therefore, our mixture of antigens had activated the macrophages locally and made them competent for clearing M. leprae.’”


“Fifteen patients with borderline leprosy who developed ‘reversal’ reactions were studied from the inception of treatment. Thirteen showed an appreciable increase in lymphocyte transformation (LT) when preparations of Mycobacterium leprae were used as antigen. The LT responses to either ‘whole’ or ‘sonicated’ preparations of the bacillus in these 15 patients and in 9 others also in reaction correlated with the clinical presentation. Those with skin disease predominating in the reaction showed an appreciable increase in LT when whole M. leprae was used as antigen. Those with nerve disease predominating showed an increase with sonicated M. leprae. In those with both skin and nerve disease there was an increase with both antigen preparations. The ratios of the LT test results (whole to sonicated M. leprae) showed highly significant differences between the three groups.”

“Mixed leucocyte cultures, from 2 normal donors, were set up in media containing human serum from one of the following sources: (a) a pool of normal group AB donors; (b) Chinese, Malay or Indian patients with untreated leprosy; (c) the same patients after effective anti-leprosy treatment; (d) control Chinese, Malay or Indian subjects. Transformation was estimated by measuring the incorporation of tritiated thymidine in the last 24 h of a 7-day culture period. Transformation was impaired in sera from untreated lepromatous patients, but was less impaired or not impaired at all in sera from treated lepromatous patients. The loss of depressive activity after treatment was more marked in Chinese and Indian than in Malay patients. Transformation was also impaired, though to a lesser extent, in sera from patients with untreated tuberculoid leprosy; it was still impaired in sera from treated tuberculoid patients. There was no evidence of specificity in impairment of mixed lymphocyte reactivity and lymphocytotoxic antibodies appeared to play no role. The incidence of hepatitis B antigen and antibody and of anti-nuclear factor were not notably high.”


“The serum immunoglobulins were determined by the single radial diffusion method in 147 cases of leprosy, 49 cases with lepromatous and 98 cases with tuberculoid type. The cases were divided into 3 groups corresponding to the duration of disease. No significant alteration of serum immunoglobulin G, A, M level was demonstrated in all of the tuberculoid patients and in lepromatous cases with the disease than 5 years, but a slight rise in IgA level was found in this group. There was a significant increase in all of the 3 classes of immunoglobulin in lepromatous patients who had had the disease more than 5 years. This finding suggested that the duration of the disease may be one of the factors affecting the variation in serum immunoglobulin level in lepromatous patients.”

3. CLINICAL ASPECTS


“Five instances of lepromatous leprosy involving lesions of the larynx were encountered among a series of 280 laryngeal lesions. These are briefly described as involving the epiglottis in all cases, vocal cords in 2, and extension into the pyriform fossa in 1 instance.”


“The index branch of the radial cutaneous nerve has been demonstrated as a constant nerve which can be readily biopsied under local anesthesia and yields a nerve which is of suitable size for quantitative and qualitative studies both by light and electron microscopy. It supplies a limited but constant area where the sensory loss does not disturb the patient.

“Definite ultrastructural changes have been demonstrated in the clinically normal nerves of leprosy patients. These nerves have also revealed a loss in the small myelinated and unmyelinated fibers, which correlated with the common clinical findings of the absence of
sweating and dissociated sensory loss in this disease. Gross damage has been encountered in nerves which showed only early signs of clinical damage even with refined methods of sensory testing. These nerves would have passed as normal on routine testing by No. 5 nylon. Regeneration of small fibers was noted following loss of large size fibers. “Nerve conduction velocity may be a useful tool in early diagnosis.”

4. THERAPY

The acedapsone prophylaxis-treatment programme in the Ponape District of Micronesia continues to provide useful data. The fourth annual post-treatment survey here reported indicates that the earlier intensive campaign has resulted in a declining incidence of new cases of leprosy. However, lapses from regularity of treatment and the emergence of sulphone resistance have complicated the issue. A few new infections have occurred from these cases. However, the main thesis of the exercise has been confirmed, that is, that susceptible individuals exposed to infection from patients suffering from multibacillary forms of leprosy may be protected by 15 injections of acedapsone given over a period of 3 years.


S. G. Browne

5. EPIDEMIOLOGY, PREVENTION AND CONTROL

“During the school year June 1973 to April 1974, 85% of the pupils attending 380 schools in Gudiyatham Taluk of Tamil Nadu were examined for evidence of leprosy. Among the 76891 children examined, 217 new cases of leprosy were detected. There was no case of lepromatous leprosy in this group. All except two borderline cases were either indeterminate or tuberculoid. In endemic areas repeated annual examination of the school going population will help considerably in the control of the disease and in educating the people. The results of school surveys carried out in some Indian cities are also included for comparison.”


Probably introduced into the island by African slaves, leprosy today is prevalent in all districts of Martinique, affecting 5.1 males per 10,000 and 2.45 females.

The authors analyse [rather superficially] the main findings in the records of 844 patients. They report that in 60% the disease showed itself before the age of 30 years, that multiple cases (251) occurred in 109 families, and that no instance of conjugal leprosy was discovered. They do not pretend that their figures are complete, a large proportion of patients admitting that they had had the disease for over 5 years before seeking treatment. Forty-seven per cent of patients are said to have lepromatous leprosy, but leprous rhinitis is quite rare. The standard treatment for three-quarters of the patients is dapsone; the remainder are given a sulphonamide.

S. G. Browne

“The paper is based on a longitudinal study of a geographically limited rural community highly endemic for leprosy. The entire population in the community numbering about 8000 was followed up every year for over 6 years, including cases of leprosy that occurred. Mean incidence of tuberculoid leprosy was found to be 10.6 per 1000 per year. Intensive follow-up of newly detected cases revealed that regression of disease among tuberculoid leprosy cases was a very common feature, the mean inactivation rate being 10.9% per year. Inactivation rates were not influenced by either age or sex. Inactivation rates were comparatively low when more than one part of the body was affected, or when the patient had more than two patches, or when he had involvement or nerve trunks. Inactivation rates were not influenced by treatment, the bulk of inactivation being spontaneous. The epidemiological significance of the findings with regard to leprosy control is reviewed.


After describing the different types of medical auxiliary carrying out essential work in the anti-leprosy campaigns in the tropics and subtropics, the author draws on his experience in the former Belgian Congo in the training and deployment of the most valuable of them all—the polycompetent auxiliary—and gives a detailed account of the curriculum and of the type of service such a qualified auxiliary can render.

*W. H. Jopling*

### 6. REHABILITATION AND SOCIAL ASPECTS


The authors examine the social reactions to leprosy elicited in a group of 40 patients from a rural area in Tamil Nadu, India, and attempt to draw general conclusions from their data.

In spite of the low degree of physical disability in the group, social rejection, marriage difficulties and economic handicap were quite marked. The prevailing attitudes to the disease itself and to its victims provide a useful background to the study of the actual experiences of sufferers themselves. The stigma of leprosy and social rejection are more evident in the educated and the more affluent classes of society. Physicians as well as social agencies could do much to break down the walls of prejudice and ignorance that still surround leprosy in the rural areas of a country like India.

*S. G. Browne*

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