CURRENT LITERATURE

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General

Leprologia 15 (1970) 63-94.

In this issue Wilkinson and Calori report that the respiratory quotient is a valuable index of greater cellular activity in patients treated with hyperbaric oxygen. In most cases, duration of period of treatment as well as the number of treatments was factorial. Higher indices were obtained less frequently in patients who had received previous antileprotic drugs. Wilkinson also found rather little significant differences in tuberculoid cases. Ribichini and Gimenez studied histologic preparations using PAS stains, as well as Alcian blue, concluding that histochemical methods are valuable in showing the degradation of bacilli, which may be masked if paraffins are not completely removed. Gimenez *et al* report on Argentine experiences with the "sanitarium-colony" as a factor in the control of leprosy, suggesting that it might well develop into a general hospital for transmittable diseases. Gimenez, Waisman *et al* report that resistance of *M. leprae* to antileprosy drugs has developed to the point that it poses a serious problem which should be studied by leprologists on a nationwide basis.—G. L. Fite

Leprologia 15 (1970) 1-62.

This issue has an editorial by L. M. B. referring to an article in Lancet (1870) entitled, "Dr. Beauperthuy's method of curing leprosy," which expressed essentially a modern humanitarian attitude. Marottoli and Gonzalez del Cerro discuss Dr. René Borsani's contributions to physical therapy in leprosy. Rotberg pursues further the prophylactic values of use of the term "Hanseniasis" as the official name for leprosy. Manzi *et al* present a broad study of the psychologic effects of leprosy on the patient, especially from the sociologic viewpoint and group attitudes. Arcuri *et al* illustrate a case of histoid leprosy. Campaigns against leprosy in the State of Entre Rios are given by Darchez, and a somewhat similar account of leprosy programming in the province of Santa Fe is offered by Vaccaro *et al*. Manzi and Dupont discuss treatment of plantar ulcers in leprosy with local application of a phenolated hydrocarbon mixture which gave fairly good results. The issue closes with a short preliminary note on sulfamethoxazole and trimthoprim in the treatment of leprosy.—G. L. Fite

Rees, R. J. W. and Waters, M. F. R. Recent trends in leprosy research. Brit. Med. Bull. **28** (1972) 16-21.

As the title indicates, this is a review which offers three principal groupings. The clinicopathological aspects are covered briefly. Experimental leprosy in the mouse receives broad coverage of its applications to bacteriology and chemotherapy. The chapter on clinical research emphasizes the increasing interest in immunologic study. A fourth note of BCG vaccination prefers further waiting for results before final assessment.—G. L. Fite

Zorin, P. M. On the provoking effect of iodobromic waters in leprosy. Vestn. Derm. Vener. **46** (1972) 66-68.

A 58 year old woman who started balneological treatment for hypertension of II A degree and nonspecific polyarthritis, developed numerous spots and plaques which were diagnosed as leprosy of the lepromatous type after five iodobromic baths. The diagnosis was subsequently confirmed by bacterioscopic, immunologic and histopath-

ologic examinations. A similar case had been observed within the previous two years but its nature had not been recognized. Before admission to balneological therapy, manifestations of leprosy were absent. As a result of resorption from the bath of iodine to which patients with leprosy are particularly sensitive, the patient developed clinical exacerbation of leprosy. Physicians of resorts and local therapeutic baths should carefully study all patients arriving for treatment in order not to overlook patients with leprosy.—(*Adapted from* author's summary)

Clinical Sciences

Saha, N., Wong, H. B., Banerjee, B. and Wong, M. O. Distribution of ABO blood groups, G6PD deficiency, and abnormal hemoglobins in leprosy. J. Med. Genet. **8** (1971) 315-316.

Four hundred and fifty-nine Chinese patients of both sexes suffering from leprosy were investigated for distribution of ABO blood groups, G6PD deficiency, and abnormal hemoglobins. There was no significant association between these genetic markers and leprosy, nor any difference of frequency distribution between lepromatous and nonlepromatous patients.—(*From* Trop. Dis. Bull.)

Sohi, A. S., Kandhari, K. C., and Nauniahal Singh. Motor nerve conduction studies in leprosy. Int. J. Derm. **10** (1971) 151-155.

The authors studied motor nerve conduction velocities of ulnar, posterior tibial and common peroneal nerves bilaterally in 30 patients, of which 12 were lepromatous cases, 6 dimorphous, 5 tuberculoid, 5 "neuritic" and 2 "maculoanesthetic." Sixteen controls were also studied. Reduction in velocity was present to some measure in all leprosy cases, especially in those in which the nerves were maximally involved clinically. Some unaffected nerves also showed decreased conduction. Alterations were exaggerated during reactive states.—G. L. Fite

Van Droogenbroeck, J. B. A. The surgical treatment of lower facial palsy in leprosy. Ann. Soc. Belg. Med. Trop. **50** (1970) 653-687.

Upper and lower facial palsies, both unilateral and bilateral, and even nearly complete facial paralysis, are not exceptional in the Far East.

It is possible to rehabilitate patients suffering from these paralysis, according to the nature of the individual case, by isolated or combined transfers of temporalis and masseter muscles (examples are given).

Although our work is essentially clinical, its aim being the treatment and rehabilitation of patients, there can be no doubt that there is much room for research in fields of leprosy and lower facial palsy.—(*From* Trop. Dis. Bull.)

Warren, Grace. Tarsal bone disintegration in leprosy. J. Bone Joint Surg. **53B** (1971) 688-695.

After reviewing the destructive effects of osteoporosis, weight-bearing and minor trauma, with resulting stress fracture, the author emphasizes the necessity for individually planned plaster support, with adequate immobilization until the bone lesions are completely healed. Most patients can be treated to ensure a useful foot. Amputation is the mark of neglect, and early treatment of this progressive disorder the path to minimal deformity.—G. L. Fite

Experimental Leprosy

Pizarro, E. Experimental infection of mice with *Mycobacterium leprae hominis:* additional data. Int. J. Derm. **9** (1970) 215-219.

This article appears to be a summary of the author's work already published in Spanish (Nota de Laboratorio, Revta Invest. Salud Publ. **26** [1966] 183). He has inoculated material from patients with leprosy into mice, firstly intraperitoneally "so we could be sure that we were not dealing with murine leprosy," and three months later, "some suspensions were reinoculated in the foot pads. With some of the animals ... lesions were found at a distance from the inoculation site." Two photographs of mice are described as showing ear lesions from which were obtained acid-fast bacilli, "which did not grow in any of the media used for mycobacteria." Acid-fast bacilli were sometimes found in "ganglia," and lesions on the "muzzle and around the eyes." Paramethasone, applied to the foot pad before the inoculation of *Mycobacterium leprae,* did not result in a "reduction of the incubation time."—C. S. Goodwin (*From* Trop. Dis. Bull.)

Shepard, C. C. The first decade in experimental leprosy. Bull. WHO **44** (1971) 821-827.

Considerable developments have occurred in the application of the method for growing *Mycobacterium leprae* in the mouse foot pad since it was first described about ten years ago. The method has been used to study growth curves and histology in normal and in thymectomized irradiated mice, to identify supposed isolates of *M. leprae* that have been made in tissue-culture or in nonliving media, to evaluate tests of experimental vaccines, to investigate applications to clinical investigations (the loss of infectivity during chemotherapy as a means of monitoring a drug trial, the demonstration of drug-resistance, and the clinical problem of the patient who responds poorly to therapy), and to study new drugs—e.g., dapsone, acedapsone, clofazimine, and rifampicin.—Author's Summary

Microbiology

Nakamura, M. Elongation of *M. lepraemurium in vitro:* experiment performed by using Boiden meshcement slides. La Lepro **41** (1972) 7-10. (In Japanese, English summary)

Boiden mesh cement coated glass slides (BM slides) were used for smears of *M. lepraemurium* which were cultivated in the medium for elongation *in vitro.* It was obvious that the BM slides had the following advantages for the experiments on elongation of *M. lepraemurium*: 1) the smear spread easily on the BM slides because of their hydrophilia; 2) the slides held the materials even if they contained much tissue protein; and 3) slight elongations of *M. lepraemurium* were detectable on the BM slides even when they were not seen on conventional slides. Therefore, the BM slide is recommended as a tool in experiments on elongation of *M. lepraemurium in vitro.*—(*Adapted from* author's summary).

Ogawa, T. and Motomura, K. Studies on murine leprosy bacillus. I. Attempt to cultivate *in vitro* the Hawaiian strain of *Mycobacterium lepraemurium.* Kitasato Arch. Exp. Med. **43** (1970) 21-36.

Infected male mice of the dd-N strain were sacrificed at different intervals. These mice had been injected intravenously or subcutaneously with bacterial suspension prepared from infected tissues of long-standing, or from a primary culture. Various organs or tissues including local lesion and regional lymph nodes were taken aseptically. Each was ground in a mortar to make a homogenate which was then, with or without decontamination treatment, inoculated by loop on 1% egg yolk medium principally, and also on 3% and 1% Ogawa's

egg media. Incubation was done at 37°C for three months and the tubes were classified as "positive" if visible colony growth developed on the surface of the medium.

None of the tissues from mice of one to four months' duration proved positive irrespective of the media employed. A number of the specimens, however, containing enormous acid-fast bacilli, all of which were derived from the group of mice of five to eight months' duration, were culture positive. Of 22 specimens inoculated on 1% egg yolk slant, 14 specimens or 64% were positive and one in four specimens inoculated on 3% Ogawa's egg slant proved positive. Those specimens with an inferior number of bacilli were all negative. The 1% Ogawa's egg medium, commonly used for cultivation of tubercle bacilli, failed entirely to support the growth of the organisms. The colony growth of the organisms was rough, slightly moist, and light yellow.

At present, the fourth generations are growing and their growth, similar to that of the primary cultures, is so slow that two to three months' time has been necessary for most of them to be clearly distinguished as positive. When smeared and stained with Ziehl-Neelsen method, such colony growth revealed strong acid-fast bacilli arranged singly or, characteristically, in a web formation. Growth occurs at 37°C, but not at 22°C or at 45°C. Reproduction test of the disease in mice was favorable. These morphological and cultural characteristics are common to all of the cultures so far isolated.—(*Adapted from* authors' summary)

Ogawa, T. and Motomura, K. Studies on murine leprosy bacillus. II. Further characteristics of slow growing acid-fast organisms isolated from experimental mice with the Hawaiian strain and an investigation of the 1% egg yolk medium. La Lepro **40** (1971) 8-15. (In Japanese, English summary)

In a previous communication some characteristics of the acid-fast organisms which had been isolated from mice infected intravenously and subcutaneously with the Hawaiian strain of *M. lepraemurium* were reported. The present paper describes some further differential characteristics of the organisms and an experimental study of the role of ingredients of the egg yolk medium.

Characteristics of the acid-fast organisms grown *in vitro*: (a) Growth response to the media for cultivation of mycobacteria. Various media such as nutrient agar, glycerol agar, Kirchner's serum agar, modified Kirchner's No. 3 agar, blood No. 1 and No. 2 agars, Dubos Tween-albumin liquid medium, and 1% Ogawa egg medium were tested for their abilities to support macroscopic growth of the organisms which had been subcultured on the egg yolk medium. All of them but the standard medium proved insufficient for supporting the growth of 39 cultures employed (Table 1); (b) The length of the bacilli. The bacillary smears prepared from the growth of cultures and stained by Ziehl-Neelsen method were subjected to a measurement of length by light microscopy (450 x mag.) with a scale in the ocular. The average for the maximum and the minimum length of the bacilli in 21 cultures examined was 10.9μ and 2.4μ respectively. Thus, the bacilli grown *in vitro* were found to be about twice as long as the bacilli in the tissues of mice infected intravenously with a primary culture of the organisms six months before; (c) Catalase. The test on five cultures revealed that the organisms were weakly positive for catalase to the same extent as *M. tuberculosis*. And atypical mycobacteria showed higher activities than those organisms above.

The 1% egg yolk medium: This medium was used as the standard to examine the effect of removal of its ingredient(s) on *in vitro* growth of the organisms. Several modified egg yolk slants were tested for such purpose using 80 cultures of the organisms. Results in Table 5 showed that out of 13 cultures inoculated, 10 cultures or 77% were positive by the use of the standard medium, whereas only 3, i.e. 23% were positive by the one deprived of phosphate, 7 cultures or 54% positive without glutamate, but none without glycerol. An egg yolk saline medium (2:1) containing no glycerol was also found to be useless. It was concluded that the only medium superior to the 1% egg yolk medium was the one deprived of malachite-green as all the other

modified media gave results either negative or inferior to those of the standard one.

The results obtained above also suggest that the acid-fast organisms which have been isolated from infected mice with the Hawaiian strain and subcultured continuously on the 1% egg yolk medium do not belong to *M. tuberculosis*, atypical mycobacteria, *M. paratuberculosis*, nor to *M. microti*. It may be suggestive of murine leprosy bacillus.—(*Adapted from* authors' summary)

Ogawa, T. and Motomura, K. Studies on murine leprosy bacillus. III. Effect of treatment in isolation culture on the invasion of saprophyte and growth of supposedly Hawaiian strain of *Mycobacterium lepraemurium*. Kitasato Arch. Exp. Med. **44** (1971) 1-11.

The specimens for culture inoculation consisted of visceral organs, superficial lymph nodes and local lesion obtained aseptically from mice of the dd-N strain previously infected by Hawaiian strain bacilli. Culture specimens were prepared as follows.

1) Untreated. Specimens were ground in a mortar and were inoculated by smearing.

2) Treatment with 1% NaOH solution. Small portion of 1% NaOH solution was added to the specimen prepared by (1) and was homogenized and inoculated by smearing.

3) Treatment with 1% H_2SO_4 solution. Instead of 1% NaOH solution used in (2), 1% H_2SO_4 solution was used. Other process followed.

4) Treatment with 1% NaOH solution and ten times diluted. Specimens were weighed beforehand and were ground, ten times 1% NaOH solution was added and homogenized, and 0.1 ml each of the homogenate was inoculated.

5) Neutralizing treatment. After neutralizing the homogenate prepared in (4) by addition of 2% HCl solution, 0.1 ml each of them was inoculated.

Two each of the above mentioned preliminary treatments and inoculation were conducted for each specimen at the same time. The homogenate was inoculated onto 1% egg yolk media on which the Hawaiian strain grows and onto 1% Ogawa's whole egg media where the Hawaiian strain does not grow. They were then incubated for more than three months at 37°C, and the contamination rate of the saprophyte and positive rate of the Hawaiian strain were compared. The results were as follows.

A. Contamination rate:

In 1% egg yolk media by (1), there was contamination in about half or more than half the number of media used; by (2) contamination decreased to about one-sixth that of (1). There was no big difference between (2) and (3), and in (4) and (5) invasion of the saprophyte was not found. Also, the contamination rate was generally smaller in 1% Ogawa's whole egg media compared to 1% egg yolk media.

B. Positive rate of the Hawaiian strain:

Excluding the facts that it was sometimes assumed that due to invasion of a small amount of the saprophyte the Hawaiian strain did not grow, and that the positive rate of (3) was as small as that of (2), no big difference was found in the positive rate of those preliminary treatments. Also, when the process of growth of the colonies was observed, it was assumed that those solutions used for preliminary treatments had slightly disturbing effects on the growth.—(*Adapted from* authors' summary)

Ogawa, T. and Motomura, K. Studies on murine leprosy bacillus. IV. Attempt to cultivate *in vitro* the Hawaiian strain of *Mycobacterium lepraemurium*. The further report on primary *in vitro* isolation, subcultivation, reproduction test of the disease in mice of slow growing acid-fast organisms, supposedly murine leprosy bacillus. Kitasato Arch. Exp. Med. **44** (1971) 33-49.

We state further the results of the isolated culture, subculture and reproduction tests of the Hawaiian strain of *Mycobacterium lepraemurium*.

1) Isolated culture and subculture. The method is almost the same as in Report I. As for the result of the isolated culture, out of 19 cases, none of the specimens from mice of one to four months' duration

proved positive. In specimens from mice of five to eleven months' duration, 6 cases (15%) of 40 in nontreated ones, 23 cases (25%) out of 92 in the group treated by 1% NaOH solution, and 3 cases (25%) out of 12 in the group treated by 1% sulfuric acid, proved positive. When the result was classified by number of bacilli contained in inoculated specimens, as the number of bacilli increased, the positive rate became better. As for the subculture, at present, the eighth generation is still going on.

2) Reproduction test. The test was made with the primary culture and the third subculture of Hawaiian strain-like acid-fast bacilli. Inoculated into mice of the dd-N strain were 0.2 mg from the subcutaneous infection and 0.1 mg from the intravenous infection. The infected mice were sacrificed at varied intervals, autopsied, and examined following Report I. The result revealed that in both strains of the primary culture and the third subculture, lesions were evident macroscopically and histopathologically. In the case of subcutaneous infection, the lesions were slight, but generally highly advanced in the intravenous infection. Also, the subcutaneous nodules were evident at the site of inoculation of the subcutaneous infection. These lesions progressed during the course of the infection. Also, positive cultures were obtained from the spleen and the local lesion of subcutaneously infected mice, and from the spleen, liver and lungs of mice of long duration of the intravenous infection. Morphologic and cultural characteristics of the organisms thus obtained were the same as that of the original culture employed for injection of the test.—(*Adapted from* authors' summary)

Ridley, M. J. and Ridley, D. S. Stain techniques and the morphology of *Mycobacterium leprae*. Leprosy Rev. **42** (1971) 88-95.

Five methods of staining *Mycobacterium leprae* are analyzed, and for full details of the technics the original paper should be consulted. Skin biopsy specimens were obtained from 11 patients suffering from lepromatous leprosy, and 50 impression smears were made from each biopsy. From ten patients with few bacilli in their skin, two scraped-incision smears were obtained from each of "six to eight sites." Three identical smears were stained by each of the five methods; one smear was examined immediately, one after 24 hours and the third after rinsing in acid-alcohol.

In the Ziehl-Neelen (Z-N) method, heat-fixed smears were stained with carbol-fuchsin at temperatures of 60°, 50°, 42°, 37° and 22°C, and staining times of 5 minutes, 15 minutes, and 18 hours. Decolorization was performed with 1% acid-alcohol for 3 minutes, or 10% sulphuric acid for 3, 10, or 20 minutes, for 25% sulphuric acid for 30 seconds or 5 or 15 minutes.

Shepard's technic with the use of formalin vapor fixation and phenol gel at 22° and 42° was employed, and another of Shepard's methods in which the gel was never allowed to dry and in which the phenol gel was replaced by 0.5% phenol was also employed.

The last two methods discussed were Aubert's technic in which a carbol-fuchsin-Tween-80 mixture is used and a fluorescent method.

The percentage of evenly-stained bacilli, the Morphological Index (MI) and the Bacteriological Index (BI) were estimated for each smear. There were two main groups of smears, those with a high MI and those with a low MI.

With the Z-N method, when the carbol-fuchsin was heated at higher temperatures, the MI was higher, this being particularly pronounced in the group with a low MI. In the high MI group, after staining for 15 minutes at 22°, the mean MI was 41, and at 60° the mean MI was 62. In the low MI group at 22°, the mean MI was 1.2, and at 60° it was 11. Decoloration with different agents did not affect the MI if the staining was for 15 minutes at a high temperature. Three percent acid-alcohol was too strong for staining at 22°C. With formalin fixation, the low MI's were consistent. Shepard's method with wet phenol gel gave a higher MI. Tween-80 was unsatisfactory. With the fluorescent method, the MI was exceptionally high but contaminants could be easily confused for *M. leprae*. The authors recommend that carbol-fuchsin be heated until

the "stain just begins to steam." With a staining time of 15 minutes the best MI is obtained. They emphasize that for drug trials standardization of technic is important.—C. S. Goodwin (*Adapted from* Trop. Dis. Bull.)

Ridley, D. S. The SFG (Solid, Fragmented, Granular) Index for bacterial morphology. Leprosy Rev. **42** (1971) 96-97.

The index described in this paper is based on the granularity index (Ridley, Trop. Dis. Bull. **58** (1961) 462) but the values are "inverted" so that 10 indicates that all the bacilli are evenly stained and 0 that all bacilli are "granular." Thus, the "Solid, Fragmented, Granular" (SFG) Index is brought into line with the Morphological Index (MI). "Solid" bacilli are evenly stained; in "fragmented" bacilli "the acid-fast substance is interrupted at one or more points, but at least one fragment displays an elongated form; also single very short rods"; and granular bacilli are "round granules either in line or in clumps." The author avers that "approximate ratios can be estimated almost at a glance." In a "smear" from a patient a value is assigned to the bacilli of each class: 2 if they appear numerous (over 20% of all bacilli); 1 if few (1-20%) or 0 (if less than 1%). Thus the relative proportion of bacilli in the three classes SFG (in this order) are represented by one of the permutations of 2-1-0. These combinations have been placed in order of descending granularity from 2-0-0 (all solid), to 0-0-2 (all granular), to give an index of 10 grades. "The order is not obvious," and for full details the original paper should be consulted. Thus, 2-2-0 is grade 8, 2-1-1 and 1-2-0 are both grade 7, 1-2-1 and 2-2-2 are both grade 5, and 1-1-2 and 0-2-1 are both grade 3. If several smears are available the mean index is taken. "The SFG Index cannot be directly equated with the MI, but it has been found entirely adequate for its purpose by clinicians."—C. S. Goodwin (*Adapted from* Trop. Dis. Bull.)

Rightsel, W. A. and Wiygul, W. C. Growth of *Mycobacterium lepraemurium* in cell-impermeable diffusion chambers. Infec. & Immunity **3** (1971) 127-132.

Successful growth of *Mycobacterium lepraemurium* has been acheved by use of a specialized diffusion chamber technic. The cell-impermeable porous chambers were maintained in animals for periods up to 50 days with and without macrophages and LM cells. A generation time of six to eight days was found for the acid-fast bacilli in chambers containing macrophages when maintained in the mouse. Also, cell-free chambers maintained in the mouse gave a generation time of 11 days for *M. lepraemurium*. There was no doubt that chambers maintained in a susceptible host provided greater yields of bacilli than chambers maintained in a nonsusceptible host such as the guinea pig. In fact, better yields were obtained when the chambers were maintained in monolayer Petri plate cultures of mouse peritoneal macrophages than when held in the guinea pig. The most pertinent observation was that living cells are not essential for growth of *M. lepraemurium*, and the results suggest that multiplication can occur in a cell-free environment within a susceptible host. These studies give evidence that the use of porous chambers has promising possibilities for further investigations on the cultivation of other fastidious mycobacteria.—(*From* Trop. Dis. Bull.)

Sasaki, Masako. On chemistry and biology of mycobacterial lipid fractions. Sci. Rep. Res. Inst. Tohoku Univ. Series C **17** (1970) 30-59.

Crude phospholipid from P6 (a scotochromogen) extracted with chloroform/methanol (2:1) yielded three major fractions. These fractions were named Fraction I, Fraction II and Fraction III on a thin-layer chromatogram in a decreasing order of Rf values.

Fraction I was identified as diphosphatidyl-glycerol (cardiolipin) and was active as an antigen in a microflocculation test and in a complement fixation reaction in the presence of syphilitic serum. The activity as a hapten in a complement fixation reaction of the Fraction I was al-

most identical with that of cardiolipin from beef heart. A positive agglutination reaction was observed between a suspension of latex coated with Fraction I and the sera from leprosy patients. Also a precipitation line was observed in the precipitation reaction between Fraction I and anti-mycobacterial antisera.

Fraction II was identified as phosphatidylethanolamine.

Fraction III which was identified as phosphatidylinositol monomannoside was found mainly in the fraction of cell wall. Phosphatidylinositol monomannoside was active as an antigen in a latex agglutination reaction in the presence of anti-P6 and anti-$H_{37}Rv$ antisera.

No protective effect of the three fractions against challenge infection with tubercle bacilli was observed.

The residue after centrifugation at 15,000 rpm of a 1/10/30 mixture of cardiolipin-lecithin-cholesterol complex in association with wax D from $H_{37}Rv$, when injected to rabbits, was found to produce anti-cardiolipin antibody.—Author's Summary

Shepard, C. C. and McRae, D. H. Hereditary characteristic that varies among isolates of *Mycobacterium leprae*. Infec. & Immunity 3 (1971) 121-126.

Isolates of *Mycobacterium leprae* in mouse foot pads were found to differ in two related properties, the average rate of growth between inoculation and harvest (G) and the number of bacilli in the harvest (H). For "fast" strains the median values for G were less than 25 days per generation, and the median values, for H were above $10^{6.1}$. For "slow" strains the median values for G were above 30, and the median values for H were below $10^{5.6}$. The G and H values for the 59 isolates for which data were available formed a continuous spectrum between the two extremes; there was no correlation with dapsone resistance. The fastness characteristic was stable; it did not change on passage in mice and was in agreement when more than one isolate had been made from the same patient. No important differences were apparent according to geographic origin of the infection of the patient. Histological studies showed that fast strains grew to a higher level without inducing the infiltrate of lymphocytes and macrophages that appear at the end of the logarithmic phase of growth in mouse foot pads. Although fast strains often had higher ratios of solidly staining (and presumably viable) bacilli in the inoculum, the fast-slow difference was not accounted for by the solid ratio. Slow strains differed from fast by having longer times until harvest and by having fewer generations of growth, even when their frequently lower solid ratios were taken into account.—(*From* Trop. Dis. Bull.)

Shu, C. K., Chung, S. L. and Lee, S. I. The changes of Bacillary and Granularity Indices of *Mycobacterium leprae* under DDS therapy. Kor. J. Derm. 9 (1971) 3-8.

The authors investigated serial changes of Bacillary and Granularity Indices from 49 previously nontreated lepromatous leprosy patients under DDS therapy during a 24-month period, and the following results were obtained.

1. Pretreatment Bacillary Index was highest on eyebrows, chins, ear lobes, arms, legs, and backs, in decreasing order.

The proportion of fall of BI during therapy showed similar tendencies in each site of smears; the average decrease being 1.2 in the first year and 0.8 in the second year.

2. The average Granularity Index before therapy was 2.5, the rise of G.I. was rapid during first 12 months, slower during next 6 months, and no significant changes were seen during last 6 months.

3. The changes of G.I. were faster and more sensitive to therapy than that of B.I. Therefore, it seems more valuable assessing the response of therapy, drug resistance, prognosis, etc.

4. Three hundred milligrams of DDS per week appear to be sufficient for maintaining the therapeutic dosage.—(*Adapted from* Kor. Med. Abstr.)

Sula, L. and Dubina, J. Cultivation of the Douglas strain of *Mycobacterium lepraemurium* in continuous culture. Bull. WHO **45** (1971) 209-212.

A liquid medium prepared from human placenta was inoculated with 10^9 *Mycobacterium lepraemurium* and incubated at 37°C for "1 to 8 months." As "visible colonies" were not observed the medium was centrifuged and the deposit was used for "continuous cultures." A drawing shows the continuous culture apparatus and it is mentioned in the results that the medium was changed weekly for one month and then monthly. Three electron microscope photographs show a long bacillus and incomplete binary fission.—C. S. Goodwin (*Adapted from* Trop. Dis. Bull.)

Tomita, T., Ito, T., Minato, M. and Kishi, Y. Filtration of *M. lepraemurium* through millipore filter. La Lepro **39** (1970) 268-271. (In Japanese, English summary)

Bacillary suspension of *M. lepraemurium* was filtered through millipore filter type HA (pore size $0.45\mu \pm 0.02\mu$), type PH (pore size $0.30\mu \pm 0.02\mu$), type GS (pore size $0.22\mu \pm 0.02\mu$) and type VC (pore size $0.10\mu \pm 0.008\mu$) respectively under a pressure of 20 mm Hg. Acid-fast bacilli in filtrates were examined microscopically after ultracentrifugation (20,000 rpm, 45 min.), and infectivity of filtrates was also examined by subcutaneous inoculation to mice.

Acid-fast bacilli were observed only in the filtrate through HA filter, and only this filtrate showed infectivity to mice. In the other filtrates which were not observed, acid-fact bacilli developed no murine leproma in mice.

Bacillary concentration of filtrate through HA filter was calculated as about 10^4/ml by means of bacterial count and infectivity test by tenfold dilution method.

It was concluded that it is needless to suppose the existence of a special filtrable form of *M. lepraemurium*.—(*Adapted from* authors' summary)

Uchida, M. and Ogawa, T. Some experiments on cultivation of *Mycobacterium lepraemurium*. La Lepro **40** (1971) 57-61.

Uchida performed this experiment to test the culture method of rat leprosy bacilli which was reported previously by Ogawa.

Three of dd-N strain and two C3H strain mice were sacrificed seven to eleven months after being infected subcutaneously with the Hawaiian strain of rat leprosy bacilli, and subcutaneous nodules, lymph nodes and various organs were isolated aseptically as culture specimens, of which a total of 30 were taken. Each specimen was pre-treated with 1% NaOH solution and inoculated on 1% egg-yolk media as well as on 1% Ogawa media (whole egg media), which was then incubated at 37°C. Three to five months later, development of macroscopical colonies were seen only on the egg-yolk media in 47.8% of inoculations, namely 11 of 23 inoculations, while seven inoculations were excluded because of contaminations. The colonies were thin, yellow and slightly moist. In Ziel-Neelsen staining, the bacilli were tubercle-like but frequently with a more elongated appearance. Though investigations of the properties or reproduction tests of these bacilli have not been performed yet, it is assumed that to date these were quite similar to the acid-fast bacilli which were cultured by Ogawa previously. At present, the colonies have developed successfully in five successive cultures.

The results described above are much the same as the ones obtained by Ogawa. (The experiment was performed by Uchida independently of Ogawa, but since Uchida fell ill, the results were described by Ogawa.)—(*Adapted from* authors' summary)

Immuno-Pathology

Wager, O. Immunological aspects of leprosy with special reference to auto-immune diseases. Bull. WHO **41** (1969) 793-804.

Leprosy, particularly lepromatous leprosy, is associated with a multitude of (auto) immune aberrations, and its clinical features also have much in common with

the collagen diseases. Immunopathological studies of the two groups of diseases may thus elucidate the basis mechanisms of both.

The reported evidence for a genetic hyporeactivity of cell-mediated immunity in lepromatous subjects is reviewed; most, but not all, of the findings fit such a hypothesis well. The possibility remains that the observed hyporeactivities may be secondary to direct effects of *Mycobacterium leprae.* Evidence for a general hyperreactivity of the antibody-mediated immunity in lepromatous leprosy is then reviewed and considered to be fragmentary. However, immune complexes containing auto-antibodies may be pathogenic. In auto-immune diseases and leprosy, the existence of pathogenic immune complexes is indirectly suggested by mixed cryoglobulinaemia and further by a number of other features reviewed in this article.—G. R. F. Hilson (*From* Trop. Dis. Bull.)

Turk, J. L. Cell-mediated immunological processes in leprosy. Bull. WHO **41** (1969) 779-792.

This is a valuable study which should be read in full. The author points out that the clinical spectrum of leprosy can be shown to depend on the degree of the cell-mediated immune response of the host against *Mycobacterium leprae.* The response is high in tuberculoid leprosy, whereas it is virtually absent in the lepromatous form of the disease. In the latter there are also defects in other manifestations of the cell-mediated immune response, such as contact sensitivity, skin homograft rejection and the ability of lymphocytes to react *in vitro.* Possible causes of the deficiencies in cell-mediated immune responses are discussed, including the question of the apparent constitutional predisposition to leprosy.—G. R. F. Hilson (*From* Trop. Dis. Bull.)

Srinivasan, H. and Namasivayam, P. R. Does entrapment neuropathy contribute to nerve damage in leprosy? Indian J. Med. Res. **59** (1971) 1385-1391.

One hundred and ninety-two adult male patients with established lepromatous leprosy were examined to find out whether increased or diminished possibility of entrapment of the ulnar nerve influenced the occurrence of damage to the nerve. This was done in order to get an estimate of the contribution of entrapment neuropathy to nerve trunk damage in leprosy. The condition of recurrent dislocation of the ulnar nerve was equated with diminished possibility of entrapment of the nerve. Nerve damage was seen to the same extent in the dislocating and normal (not dislocating) nerves. The olecranon-medial epicondyle interval was used as the second parameter. Significant increase in the occurrence of nerve damage was seen when this interval was small (25 mm or less) when the elbow was straight and when this interval increased by 50% or more after elbow flexion, suggesting that increased possibilities of entrapment led to increased occurrence of nerve damage. It is pointed out that routine extraneural decompression of the ulnar nerve as a prophylactic measure to significantly lower the frequency of nerve damage is not likely to be successful in view of the finding that limbs at high risk formed only a small minority of the total.—Authors' Summary

Reyes, O. Aspectos histoquimicos del granuloma lepromatoso. [Histochemistry of the lepromatous granuloma.] Derm. Venez. **9** (1970) 967-973.

The English summary appended to the paper is as follows. Biopsies were taken from a number of cases of lepromatous leprosy and studied histochemically in order to determine the presence of neutral and acid mucopolysaccharides, metachromasia, amyloid substance, hemosiderin and DRN acid.

The Alcian-blue stain was positive in the amorphous masses of acid-fast bacilli found in some cases but not in the biopsies which did not show those masses. A mild degree of metachromasia was observed and both bacilli and/or the cellular membrane of the cells of the granuloma take the PAS strain with varying intensity. The Pearls', Congo-red and Feulgen stains did not reveal anything of interest. After carefully reviewing

the records of these cases no relationship could be found betwen the histological findings and the course of the disease, reactional symptoms or treatment received.—(*From* Trop. Dis. Bull.)

Ozaki, M., Furuta, M., Murakami, M., Funahashi, A., Takahashi, S., Harada, N., Ichiba, H. and Matsumoto, S. Three autopsy cases of acute Klebsiella pneumonia. La Lepro **40** (1971) 62-67.

Three autopsy cases of acute Klebsiella pneumonia were reported. Two were male leprosy patients in Komyoen Leprosarium and the other a female patient from a general hospital. All of them were over fifty. The onset of each case was sudden and they died within one week. However, physical examination of the lungs revealed mild deterioration in all cases. Their symptoms were not limited to the respiratory system.

Autopsy findings revealed typical lobar pneumonia due to *Klebsiella pneumoniae*. Infection of this gram negative bacilli did not relate to leprosy; it is the same as other bacterial pneumonia which occurs equally between leprous and nonleprous patients. The histopathologic findings of the lung showed remarkable lymphocytic infiltration rather than the polymorphonuclear leukocytic one; it was different from the description of textbooks. Infiltrating cells of pulmonary lesion were slightly different among the three cases. Old lepromata with a mild lymphocytic infiltration was seen in the liver, spleen or adrenal of the leprosy cases. Acid-fast bacilli were not found in these lesions.

Klebsiella pneumoniae is thought to exist in normal flora of the human pharynx. In our cases, leukopenia was not the cause of this infection. One leprosy patient was a heavy drinker. Alcoholism seemed to relate to etiology of the pneumonia; there had been little reference in previously reported cases in Japan. Sulfone therapy of long duration for leprosy may effect the human flora, but this was not clear in our cases. For treatment and improvement of prognosis, it is necessary to identify the bacilli and choose sensible antibiotics.—(*Adapted from* authors' summary)

Hay, J. B., Murphy, M. J., Morris, Bede and Bessis, M. C. Quantitative studies on the proliferation and differentiation of antibody-forming cells in lymph. Amer. J. Path. **66** (1972) 1-24.

The transforming cells that appear in the efferent lymph from a lymph node responding to an antigenic challenge are part of a heterogeneous population which changes as the response progresses. Some cells containing small amounts of antibody appear early in the response and these cells have the cytologic characteristics of small and medium lymphocytes. They are, however, actively synthesizing DNA. As the immune response progresses, the antibody content of the cells in lymph increases. When incubated *in vitro*, cells in lymph appearing late in the response released 20 times more antibody per cell than those appearing early in the response. Large blast cells are the predominant antibody-forming cell in lymph. At the peak of a secondary challenge with horseradish peroxidase, up to 40% of the cells in lymph may be blast cells and, of these, two-thirds may contain specific antibody. It seems probable that most if not all of the blast cells responding to the antigen are involved directly in antibody and DNA synthesis. Cells in all stages of ultrastructural differentiation, and even mature plasma cells, were found to incorporate ^{3}H-thymidine into their nuclear DNA.—Authors' Summary

Tanaka, Y. and Mori, T. Determination of putrescine and cadaverine in the serum from lepromatous leprosy patients. La Lepro **41** (1972) 1-6. (In Japanese, English summary)

Ishikawa reported that putrescine is always observed in the blood of lepromatous leprosy patients with *M. leprae* in their skin (La Lepro **36** [1967] 238). It is well-known that putrescine is one of the ptomaines and causes ptomaine poisoning. Ishikawa's report presents a serious problem confronting lepromatous leprosy patients. In this paper we described the method of determination of putrescine and cadaverine in serum and then the result of

measuring these ptomaines in the serum from lepromatous leprosy patients.

The determination of putrescine and cadaverine is as follows. Serum was extracted with n-butanol by McIntire's method. Butanol layer was acidified with 1N-HCl and then butanol was removed by lyophilization. Lyophilized residue was applied to a small column of Amberlite CG-50 according to the method of D. R. Morris with some modification.

By the use of Amberlite CG-50, putrescine and cadaverine could not be separated, but could be separated from lysine, histidine, arginine, histamine, etc. Standard curve of ninhydrin reaction showed that the concentration of putrescine or cadaverine was in proportion to the rate of ninhydrine reaction. The recovery of putrescine in normal human serum after butanol extraction and column chromatography was about 90%.

About 2 ml (varying from 1.6 to 4.5 ml) of serum from a lepromatous leprosy patient with bacilli in his skin was tested for detection of putrescine and/or cadaverine, but in no case out of seven did we find more than 0.05μ moles of putrescine and/or cadaverine in the serum.

Ishikawa obtained the serum from 5 ml of blood, so about 2 to 2.5 ml of serum was used for detection of putrescine. The quantity of serum used for determination of putrescine by Ishikawa is almost the same as that used by us. We do not believe that Ishikawa's method is far better than that used by us.

Putrescine and cadaverine are known to be formed by decarboxylation from ornithine and lysine respectively in living organisms. From our result we cannot decide whether *M. leprae* has decarboxylase activity or not, because the products of this bacillus may be metabolized to nontoxic materials by the human enzyme system to maintain the homeostasis. For example, even if the putrescine is produced, it will perhaps be oxidized by diamine oxidase in human liver. It seems necessary to measure directly decarboxylase activity in the bacillus.—(*Adapted from* authors summary)

Murphy, M. J., Hay, J. B., Morris, B. and Bessis, M. D. Ultrastructural analysis of antibody synthesis in cells from lymph and lymph nodes. Amer. J. Path. **66** (1972) 25-42.

A variety of cells containing antibody were found in lymph from sheep responding to secondary challenges with horseradish peroxidase. Antibody was present in blast cells in lymph within the perinuclear space, the endoplasmic reticulum, the Golgi apparatus and on polyribosomes. Some lymphocytes in lymph also contained antibody but in these cells it was located principally in the perinuclear space. No cells were found containing antibody distributed throughout their cytoplasm nor were any lymphocytes found with a Golgi apparatus positive for antibody. After the immune response in the lymph had died away, a population of cells containing antibody was still present in the regional lymph node. These were all plasma cells in which the antibody was present for the most part in a highly organized endoplasmic reticulum. Cells of this type were never found in the lymph. The cells containing the smallest amounts of antibody had a few discrete focal points in the perinuclear space and a few positive groups of ribosomes in their cytoplasm. The endoplasmic reticulum in some cells was filled completely with antibody, while in others positive segments were found adjacent to negative ones. The antibody in the cytoplasmic endoplasmic reticulum was in continuity with the antibody in the perinuclear space. The Golgi apparatus contained antibody in only a small proportion of the cells but when it was positive it was strongly so, suggesting that antibody was concentrated in this organelle. In some cells a positive reaction to horseradish peroxidase antibody appeared in the nucleus over the nucleoli. The significance of this finding is not known.—Authors' Summary

Mittal, M. M., Agarwal, S. C., Maheshwari, H. B. and Kumar, S. Renal lesions in leprosy. Arch. Path. **93** (1972) 8-12.

Although leprosy does not primarily affect the kidney, autopsy reports indicate

that renal disease is a common complication. The present paper, however, is almost the first biopsy study of the subject. Renal biopsy was carried out in 30 patients with leprosy (18 lepromatous and 12 nonlepromatous), 28 of whom appear to have given a history of 10-24 years' duration and only 17 of whom were on chemotherapy. In 15 of the 30 patients, biopsy revealed a variety of pathological conditions, comprising interstitial nephritis (13 cases), hypercellularity of the glomerular tuft (8 cases), tubular degeneration with protein casts (2 cases), pyelonephritis (1 case) and hyalinization of small and medium sized arteries (4 cases). Amyloid was not found in any of the biopsies (its incidence in leprosy in India is remarkably low compared with leprosy elsewhere). The other 15 patients gave normal renal biopsies.

Neither the clinical type of leprosy nor its duration or therapy appeared to be correlated in any way with renal disease, whose pathogenesis is a matter for speculation. It is suggested, however, that the nonspecific changes affecting the glomerular tuft and blood vessels might be the result of an immune mechanism.

[The lack of correlation between renal disease secondary to leprosy and the type of leprosy or history of reactions is contrary to the findings of some other workers and a little hard to accept; so also is the absence of proteinuria, especially as protein casts were seen.]—D. S. Ridley (*Adapted from* Trop. Dis. Bull.)

Chemotherapy

Opromolla, D. V. A. and De Almeida, S. C. Primeiros resultados do tratamento da lepra com kanamicina. [Treatment of leprosy with kanamycin: preliminary results.] Rev. Brasil Leprol. **87** (1970) 17-39.

Ten patients were treated with one gram of kanamycin daily for 90 days. The results were similar to those obtained with rifampin or other antibiotics, but improvement was observed as early as 30 days. In three cases, bacteriologic negativity was obtained, and evidence of the bactericidal effect of kanamycin was noted both in smears and histologic sections. *Erythema nodosum* was not a problem, but the authors encountered some deafness and recommend careful audiometric control with this drug. While not suitable for mass treatment of leprosy, especially on an outpatient basis, kanamycin was thought to have significant value in treatment of cases refractory to further improvement with other medications. Further work is suggested, to establish minimal effective doses, or those free of ototoxicity.—G. L. Fite

Otsyula, Y., Ibworo, C. and Chum, H. J. Four years' experience with dapsone as prophylaxis against leprosy. Leprosy Rev. **42** (1971) 98-100.

This is a report from the East African Leprosy Research Centre (Busia, Tororo, Uganda) of a controlled trial of dapsone in the prophylaxis of leprosy. Two districts (Samia and Bunyala locations) were chosen where a preliminary survey had shown the incidence of leprosy among school children to be similar, and dapsone was given to 3,380 children in one area (Samia), and 1,393 children in the other area (Bunyala) received no treatment. The period of the trial was from February 1963 to December 1967 (four years and ten months). During this period four children in the treated group developed leprosy (1.2 per 1000), and 13 in the nonprotected group (9.5 per 1000), an eight-fold difference which is statistically significant.

[It is unfortunate that dosage of dapsone is not mentioned, and there is a discrepancy in the text in that the period of treatment is stated to be three years and ten months.]—W. H. Jopling (*Adapted from* Trop. Dis. Bull.)

Pattyn, S. R. and Wagner, W. H. Activity of compound TH 270 (N-p-isobutoxyphenyl-N'-p'-α-pyridyl-ethyl-phenylthiourea) on the experimental infection with *Mycobacterium lepraemurium* and *Mycobacterium leprae*. Ann. Soc. Belg. Med. Trop. **52** (1972) 55-61.

The activity of a Thiourea compound (TH 270 (N-p-isobutoxyphenyl - N′ - p′ - α - pyridylethylphenylthioureum) against *M. lepraemurium* and *M. leprae* in mice was investigated.

TH 270, which has a pronounced antituberculosis activity is inactive on *M. lepraemurium* but definitely active on *M. leprae*. Cross resistance with *thiambutosine* is probable.

Information about plasma-levels obtained after oral and/or parenteral administration of TH 270 are needed to allow a better judgement of the therapeutic value of TH 270.—Authors' Summary

Prasad, B. N. Trial of high dosages of dapsone in the treatment of tuberculoid leprosy. Leprosy Rev. **42** (1971) 118-120.

The author, working in Bihar, India, treated two groups of patients suffering from tuberculoid leprosy, giving conventional doses of dapsone to one group of 100 patients (50 mg twice a week progressively increasing to 100 mg daily), and 200 mg daily to the other group of 100 patients. Treatment was given for twelve months, and for the next two years the patients were examined every four months for signs of relapse. Those on the higher dosage of dapsone improved more rapidly and there were no relapses; whereas, in the other group, eight patients relapsed within three to six months of stopping treatment. Nine patients in the high dosage group experienced side-effects (headache, loss of appetite, loss of taste, abdominal pain, anemia). No psychosis was observed.

[No details are given of the anemia encountered or of any side effects experienced by the group on the smaller dosage except to say that "five patients could not tolerate the drug." Were these patients removed from the trial? The critical reader may well wonder why there was no intolerance to the larger dosage.]—W. H. Jopling (*Adapted from* Trop. Dis. Bull.)

Russell, D. A., Shepard, C. C., McRae, D. H., Scott, G. C. and Vincin, D. R. Treatment with 4,4'-diacetyl-diaminodiphenylsulfone (DADDS) of leprosy patients in the Karimui, New Guinea. Amer. J. Trop. Med. Hyg. **20** (1971) 495-501.

The authors report on the clinical and bacteriological results of the first 750 days of treatment of 28 patients with leprosy (out of 327 in a trial reported elsewhere) who had sufficient numbers of leprosy bacilli in their skin smears for appraisal of the proportion of solidly staining bacilli (Morphological Indices) during treatment. This investigation was carried out in the Karimui region of New Guinea, and patients were given an intramuscular injection of the diacetyl derivative of dapsone (DADDS) every 75 days, each injection consisting of 225 mg for adults and 150 mg for children under the age of six years.

Morphological Indices fell to near zero in 150 days, and there was a fall in Bacterial Indices compatible with that which would have been expected from standard dapsone therapy. Clinical response and incidence of lepra reactions were also satisfactory. No drug resistance was noted, but the authors admit that continuing observation of these patients is necessary because of the small quantities of dapsone released by the depot injections (averaging 2 to 4 mg daily).—W. H. Jopling (*From* Trop. Dis. Bull.)

Satake, Y., Nakamura, K. and Imamura, K. The basic studies of chemotherapy of *p*-(p'-aminobenzenesulfonyl)-benzaldehyde thiosemicarbazone for leprosy. First report. (Satake) La Lepro **38** (1969) 227-236. Second report on metabolites of thiozamin *in vivo*. (Satake, Nakamura, Imamura) *Ibid.*, 237-245.

The compound *p*-(p'-aminobenzenesulfonyl)-benzaldehyde thiosemicarbazone was synthesized by Satake and has the designated name of thiozamin. Part of its structure is similar to sulphones and part to the thiosemicarbazones. Thiozamin "up to a 1 to 5,000 diluted solution . . . was effective in checking the growth" of *Mycobacterium tuberculosis* H37Rv, but it had no effect on *Staphylococcus albus* or "colibacillus." The growth of the BCG strain of *M. bovis* was inhibited to "about 33%" (but very few details of these antibacterial experiments are given). Thiozamin was "mixed in the

diet" of 10 mice infected with *M. lepraemurium* (but no details of the concentration of the drug or the day of infection or drug administration are given) and its effect compared with mice treated with isonicotinic acid hydrazide and untreated controls. "The sizes of their lepromas" are detailed, and thiozamine appeared to inhibit the growth of these lesions. Experiments to detect the mechanism of the antibacterial action of thiozamine on BCG bacteria failed to reveal any effect on respiration, protein synthesis or ribosome activity. In mice a dose of 1705.5 mg/kg was "very weak poisonous."

In the second paper various experiments on rabbits fed on thiozamine are reported. The urine was examined for an N-glucuronide metabolite, but none was found. By paper and thin-layer chromatography and extraction technics, some metabolites of thiozamin were found that showed "ultraviolet absorption," and these were thought to be the monoacetylated derivatives. (In view of the chemical structure of this compound it would seem likely that any antimycobacterial action is due to its sulphone and thiosemicarbazone moieties.)—C. S. Goodwin (*From* Trop. Dis. Bull.)

Satake, Y. and Ito, T. Studies on anti-leprotic agents: Basic studies on pyrazine derivatives for the chemotherapeutic agents of leprosy (First report). La Lepro **39** (1970) 26-32. (In Japanese, English summary)

The authors synthesized certain pyrazine derivatives. Each compound was employed in the experiments.

For an experiment on their antibacterial actions, the compounds were examined for their effects on *Mycobacterium tuberculosis*, $H_{37}Rv$. The results showed that Pycazid 200r<, PBS-1 10 r<, PBS-2 5 r< diluted solutions, PBS-1 and PBS-2 were effective in checking the growth of $H_{37}Rv$ but the effect of Pycazid was weak.

On acute toxicity to mice, the results of the experiment were as follows:

LD_{50}	Pycazid	150.5 mg/kg
	PBS-1	1510.0 mg/kg
	PBS-2	1540.0 mg/kg

There was no effect of the three compounds on the respiration and blood pressure of rabbits in a dosage of up to 100 ml of 0.02% solution. No effects were found on the peripheral blood vessels of rabbits to 15 ml of each solution (0.02%).

Actions on the excised toad heart; 5-10 mg/20 ml of PBS-1 and PBS-2 showed no action. But, 1 mg/20 ml of Pycazid caused an increase in amplitude lasting for minutes. The heart beat became irregular and came to a standstill at the systolic position with a dose of 20 mg/20 ml.

Actions on rabbit intestines. The actions of the compounds on the motility and tone of an excised small intestine were tested by the Magnus's method. Neither PBS-1 nor PBS-2 showed any action. The intestinal tone was barely strained with 10 mg/20 ml of Pycazid, but a gradual decrease caused by application of Pycazid in the tone of the intestine could be antagonized by after-treatment with $BaCl_2$.

The spasmogenic activity of acetylcholine was reduced remarkably with the application of Pycazid.

The pharmacological actions and toxicities of PBS-1 and PBS-2 were very weak, PBS-1 and PBS-2 were designated to be used in clinical treatment tests on leprosy.—(*Adapted from* authors' summary)

Sheskin, J. Recent experience with thalidomide in Hansen's disease. Int. J. Derm. **9** (1970) 56-58.

The author reviews briefly his 55-month experience in this field. The lepra reaction of patients with lepromatous leprosy (pregnant women excluded) responded rapidly to thalidomide in a dose of 400 mg daily with subsequent gradual reduction in dosage. Where the velocity of nerve conduction is impaired during the reaction, thalidomide rapidly restores it. In a preliminary investigation, high values for complement were found in the sera of patients in lepra reaction. These levels "generally" fell rapidly with thalidomide.

Thalidomide was ineffective as the sole treatment for leprosy in 24 patients over periods of up to 19 months. Toxic effects were not severe and never caused treatment to be stopped.

[There are no references—the reader will find fuller details elsewhere, *e.g.* Trop. Dis. Bull. **67** (1970), abstr. 2187; Internat. J. Leprosy **37** (1969) 359.]—H. V. Morgan *From* Trop. Dis. Bull.)

Sheskin, J. Cinco años de experiencia con talidomida en la lepro-reacción y el mal de Hansen. [Five years experience of thalidomide in lepra reactions of leprosy.] Med. Cutanea **4** (1970) 379-381. (In Spanish, English summary)

After five years of trials it may be stated that thalidomide is very effective in lepra reactions of the lepromatous leprosy type, but is not effective as the sole treatment for leprosy itself.

For patients who do not tolerate sulfone alone, the addition of thalidomide is recommended. The optimal dose of thalidomide was found to be 400 mg/day (6 mg per kg of body weight), and the optimal maintenance dose 100/mg day. In patients that have been under long-term treatment with corticosteroids, it is advisable to start with the optimal dose of thalidomide even before the gradual reduction of the steroids is completed.

Thalidomide might possibly play a role in reducing or even perhaps preventing neural, muscular and osseous changes following reactional neuritis. Side effects were mild and did not necessitate withdrawal of treatment. Repeated laboratory examinations of blood and the other body fluids did not reveal any deviation from normal values.—(*From* Trop. Dis. Bull.)

Sheskin, J. Influencia de la talidomida, esteroides, analgésicos y placebos sobre la velocidad de la conducción motora en la neuritis cubital reaccional. [Effect of thalidomide, steroids, analgesics and placebos on motor conduction velocity test in reactional leprosy with neuritis.] Med. Cutanea **4** (1970) 459-463. (In Spanish, English summary)

Sixty-nine motor conduction velocity test examinations were carried out in six reactional leprosy patients with neuritic symptoms. The ulnar nerve was examined before the reactional state, during the reaction without therapy, and during the following thalidomide and steroid therapy.

Following thalidomide therapy, there was a striking improvement of the MCVT, which occurred usually within forty-eight hours to one week. A similar improvement occurred following steroid therapy, but was much slower and less striking.—(*From* Trop. Dis. Bull.)

Tsutsumi, S., Sakamoto, Y. and Nakamura, K. Analytical studies on antileprous drugs. VI. On the analysis of sulfone drugs and their metabolites by thin-layer chromatography. La Lepro **39** (1970) 17-25. (In Japanese, English summary)

To find out a favorable method for the analysis of various sulfone drugs and their metabolites, the separation of 55 compounds relating to the present drugs was studied by thin-layer chromatography (TLC).

The separation of DDS, DDSO, 4, 4′-Diaminodiphenyl sulfide (DDSD) and their mono-N-acetylates is easily achieved on a thin-layer plate. When benzene/ethylacetate (2/3) was used as a developing system, the order of their Rf values was S> SO_2> SO and NH_2>$NHCOCH_3$. Also, the order was found to be R, R′: NO_2> H > $OCH_2CH_2OC_2H_5$ > NH_2NH > OH, NH_2 > $NHCOCH_3$, $NHCH_2CH_2OH$ > COOH, $NHCH_2COOH$ in the case of 4-R-$C_6H_4SO_2C_6H_4$-R′-4′. When the same solvent system was used, no clear difference was found between the Rf value of diphenyl sulfone compound (DS) and that of the corresponding diphenyl sulfonamide (DSA) or the diphenyl ethyl sufonamide (DESA), though the Rf value of the former was somewhat higher than that of the latter two.

Applying this method, a sample of human urine was examined after the oral intake of DDSO. The metabolites identified were DDSD, the mono-N-acetylate (MAcDDSD) and DDS (all of them faintly detected), and MAcDDS, unchanged DDSO, and MAcDDSO (all of them markedly detected).

It was also coincident with the result of the experiment, that the MAcDDSO was

more labile under auto-oxidation than DDSO when they were allowed to stand in ethylacetate. Therefore, it can be said that this experiment suggests the reason why a considerable portion of DDSO is found to be existent as MacDDS in the urine sample, even though this oxidation is presumed to be accelerated by TPN-linked liver microsomal function. However, in the urine sample of a rabbit, DDS, MAcDDS, MAcDDSO, and unchanged DDSO could merely be detected.

Several authors have already reported the reduction of some dialkyl sulfoxides to the corresponding sulfides by an enterobacteria or their oxidation by liver microsomes. However, no reports mentioned the reduction of diaryl sulfoxides *in vivo*.

In the second step, the separation of some water-soluble labile derivatives or conjugates of DDS was carried out to certify the main metabolites of Promin by TLC. A compound named Promin A (PrA) was prepared for this purpose, together with ^{14}C-PrA, and intraveneously injected into several rabbits. These were synthesized by sodium glucuronate (GNa), glucuronic amide (GNH_2) or GK-6^{14}C respectively, instead of glucose in the case of Promin. All of them were more labile than Promin in aqueous solution.

As a result, it was found that the main metabolites in the rabbit urine were DDS and mono-N-glucosiduronate (DDSG). This result suggested that a main metabolite of Promin might be DDS mono-N-glucoside, especially if the Rf values of DDS mono-and di-N-glucoside were compared with those of the corresponding mono- and di-N-glucosiduronate on a TLC plate, even though DDS N-glucosides were more labile than the N-glucosiduronates, and also the mono-N-glucoside as a standard material could not be isolated yet.

In spite of reports by Dr. Sweet and Dr. Bushby who had respectively presumed and maintained the existence of DDS mono-N-glucose sulfonite (mono-Promin) as a main metabolite of Promin, this predominant desulfinition was again certified in the case of PrA.

In addition, because the measured radioactivity in the rabbit urine and the plasma was calculated to be too low to explain the total activity in ^{14}C-PrA injected into the rabbit, the GK-6^{14}C freed from ^{14}C-PrA was supposed to be separately catabolized *in vivo*, though the relation between the xylulose cycle could not be explained. This result was also observed when DDSG-6-^{14}C was injected similarly into a rabbit.—(*Adapted from* authors' summary)

Tsutsumi, S., Sakamoto, Y., Nakamura, Kazuaki, Nakamura, Kazunari, Hisai, S. and Kashima, E. Studies on the improvement of antileprous drugs III. The inhibitory effect of some drugs on the growth of leprosy bacilli in the foot pads of mice (Part 1). La Lepro **39** (1970) 33-47. (In Japanese, English summary)

Reliability of the screening method employing the foot pads of mice in the search for antileprous drugs has mainly been evaluated in the use of drugs which have already been established to be effective in the treatment of leprosy, tuberculosis or malarial disease, or otherwise, and in the use of some masked compounds of DDS, which were expected to be longer-active and also lower-toxic than DDS.

In the present study the authors employed some compounds which had not been clinically tested on mycobacterial diseases, together with some of 60 compounds which have been newly synthesized. These consisted of three series. Each was given orally to the mice, mixed in the diet at concentrations of 0.1 mg or 0.5 mg/g, or injected intramuscularly in a corresponding dosage into the dorsal muscle once a week.

The strains of leprosy bacilli employed were N Aneta P_2 or B 2409 P_{12}, and the transmission was carried out to inject one of the strain into both of the hind foot pads of the experimental mice. The bacterial growth was examined by counting bacilli in each slice of the foot pad covering a period of approximately eight months. A serious deviation in the bacterial counts was observed within a group. However, in most cases, the bacterial counts in the right and the left hind foot pads of each mouse were comparable.

As exceptional cases, the results of INAH, 1314 TH, and Ethambutol were

contradictory to their respective clinical findings, and s-DDS, the chemical structure of which is similar to DDS, and especially to Promacetin, was ineffective, though the results stated in this report are merely based on the continuous method.

To elucidate the origin of such a contradition, the concentrations of these exceptional case drugs in the foot pads or in the blood of mice were measured by a radiotracer technic. Based on the results of this experiment, the relative difficulty of these drugs to arrive in the foot pads of mice was not thought to play an important part in their effectiveness in the foot pad method. Therefore, this contradiction may be caused by a difference between the mode of action of a drug against the parasitic leprosy bacilli in the macrophages of the foot pads of mice and that in the human lepra cells. The Aryl Propiolthieanilides are considered to be promising.

Subsequent to this study, various kinds of compounds untested on any infections or newly synthesized, together with numerous antibiotics, will be tested by the mouse foot pad method in the next stage—(*Adapted from* authors' summary)

Tsutsumi, S., Sakamoto, Y., Nakamura, K., Yoshino, Y. and Kunikoshi, U. Studies on the mode of action of antileprous drugs. I. Several problems on the inhibitory action of sulfones against the metabolism of mycobacteria. La Lepro 39 (1970) 48-60. (In Japanese, English summary)

With the progress of metabolic and especially of histopathologic investigations, a hypothesis develops regarding the mode of action of sulfones against leprosy bacilli which differs from that of a simple *in vitro* competition between sulfonamide and the folic acid biosynthesis by microorganisms. Because the studies on the sulfonamide-type inhibition were still in limited status in the field of mycobacteria, a series of studies were performed step by step to elucidate these problems.

In this report, the inhibitory effect of DDS against the intake of some radioactive nutrients by numerous strains of mycobacteria was examined in comparison with that of s-DDS (DDS 2-sulfonamide) and DDSA (4, 4′-diaminodiphenyl sulfonamide), together with that of some PAS derivatives; two of them were newly synthesized.

The intake inhibition of PABA was generally found in the phase of partially inhibited growth of mycobacteria. While the drugs employed, except hydrazino-PAS derivatives, showed this inhibition against all the strains employed, only DDS was effective on leprosy patients or on the growth of leprosy bacilli in the foot pads of mice. Also, this inhibition was limitedly seen in the case of Promin, even after 96 hours of incubation.

Although an inhibition of DDS against the intake of glutamate was slightly detected, the intake inhibition of tested nutrients other than PABA could not be clearly detected.

As for the PAS derivatives, PAS methylester (PASMe), whose carboxylic radical was masked, showed an intake inhibition of PABA, though it was somewhat inferior to that of PAS. Therefore, the relationship between the chemical structures of the PAS derivatives and their *in vitro* growth-inhibitory action on five strains of mycobacteria were examined, in which three strains of human tubercle bacilli showing resistance to some antituberculous drugs were included. It was found that PAS hydrazide (PASH) had the highest action even on PAS-resistant strains and that PASMe showed an effect similar to PAS. However, the derivation of the NH_2-radical of the PAS derivative into the corresponding hydrazine compound brought about a lowering of the action while, in the case of sulfones and sulfonamides, it is well-known that this derivation brings about a favorable result. IR spectrophotometric analysis showed that only PAS formed an intramolecular hydrogen bond among these PAS derivatives. Therefore, the mechanism of the *in vitro* action by PASH was thought to be different from that by PAS. Both the hydrolysis of PASMe into PAS and the decarboxylation of PAS by *M. phlei* growing in Dubos' broth nutrient fluid could not be detected.

To find out any relation between these *in vitro* results and the mode of action of sulfones *in vivo*, at the first step, the dis-

tribution of ^{3}H-labled Promin in leprous nodules of 17 leprosy patients was examined, and it was found that the effective concentrations of DDS in Promin were calculated to be only 3-4 μg at 12 hours per 1 g (wet weight) of each nodule after an injection.

Based on these results, a bacteriological reconsideration is discussed, concerning the relation between the mode of action of sulfones *in vitro* and *in vivo*.—(*Adapted from* authors' summary)

Tsutsumi, S., Sakamoto, Y., Nakamura, K., Abe, M., Minagawa, F., Yoshino, Y., Ohsawa, Y. and Sato, T. Studies on the mode of action of antileprous drugs. II. Their adsorption on the protein components of human serum. La Lepro **39** (1970) 179-192. (In Japanese, English summary)

The adsorption of antileprous agents on a protein component in leprous nodules has been examined by Tamemasa, and it was thought that this component was an albuminoid protein. It was also found that there was a proportionality between the adsorption of several agents on this component and that on human serum albumin, respectively, to the agents. However, the content of this component in the leprous nodules was not clearly indicated and only a few antileprous and antituberculous agents were put to the test in nonconjugated forms. The purposes of this study are to determine whether albumin (A) is the only main component of serum in adsorption of the drugs, and whether the hypothesis advocated by Tamemase can be employed in the search for antileprous agents.

Seventeen drugs, mainly antileprous or antituberculous agents, were tritiated by Wilzbach's method and their adsorption on a human serum sample was examined by the following four methods; (a) molecular filtration by Sephadex G-200, (b) ultrafiltration, (c) electrophoresis with a filter paper (PEP) or a cellulose acetate membrane (CAE), and (d) ultracentrifugation.

Results obtained were as follows:

(1) By method (a) it was observed that even if the main component bound drug was still A, except in the case of INAH and 1314 TH, a considerable portion of bound DDS, especially of 4, 4′-dihydrazinodiphenyl sulfone (DHDS) was detectable in the first protein pattern, the peak of which consisted of $\alpha \gg \beta G > A$.

(2) The main adsorption was found in the first pattern in the case of INAH and 1314 TH, especially in the latter. While, in the case of Ciba 1906 and sulfamethoxpyridazine, almost all of the radioactivity added to the serum was detected in the third pattern, the peak of which consisted of $A \gg \beta > \alpha G$.

(3) By method (a), it was found that mono-N-acetyl DDS (MADDS) seemed to have more affinity than DDS for the protein components in the third pattern. But in the first pattern, conversely, DDS seemed to have more affinity than MADDS for the protein components. In the case of DDS mono-N-glucoronide (DDSG), no noticeable adsorption could be detected throughout the patterns.

(4) It was found by method (a) that, in spite of the hypothesis by Tamemasa, the bound ratios of such agents as promizole, kanamycin, rifamycin, or ethambutol were not high, though all of them were known to be effective or partially effective on leprosy. Also, it was noticed that the bound ratios of PAS, which were known to be ineffective on leprosy, were comparatively higher than those of the sulfones employed when it was measured by both methods (a) and (b). All of the usual sulfonamide agents such as sulfonamide, sulfaisoxazole, and homosulfamine showed only slight adsorption in the third pattern.

(5) The result described in (1) was noticed again when each of the adsorption ratios of DDS to the several protein components of human serum measured by method (b) was compared with the corresponding values for sulfamethoxypyridazine, though the special purification of α and β G of a commercial grade was not carried out.

(6) Adsorption ratios of some agents such as DDS, promizole, sulfaisoxazole, and PAS measured by method (b) were 30-65% higher than those values correspondingly calculated by method (a). This suggested the possibility that a considerable portion

of these agents, once adsorbed on the serum are again freed from the serum during molecular filtration. Since these agents show remarkably high adsorption ratios, the ratios measured by method (b) may be comparable to the values correspondingly calculated by method (a).

(7) By the PEP method, the results described in (1) and (2) were again recognized for DHDS and Ciba 1906. However, in the case of DDS and promizole, this was not clearly demonstrated because, whether the serum was added or not, a considerable portion migrated to the anode similarly to the corresponding control samples or remained near the original lines. The slight adsorption of sulfaisoxazole and rifamycin on A found by method (a) was again noticed. Although the bound ratios of PAS to the three protein patterns in (a) were in the order of third>first and second, the adsorption on G could not be clearly detected by the PEP method. Ethambutol migrated to the cathode and no adsorption could be found.

(8) The CAE method was defective in spotting sufficient radioactivity to examine the adsorption of the agents labeled by the usual contact method on the minute protein components in the serum.

(9) As the ultracentrifugation proceeded, such components as A and α_1G gradually decreased in the lower layers in contrast to the increase of α_2, β γG. However, these changes involved an overly complicated relation to prove a delicate difference between the adsorption of an agent and that of the others. In conclusion, the following two points can be stated: (i) Because A/G gradually decreases in the lower layers, adsorption of A-affinitive type agents per mg protein may decrease more rapidly than that of G-affinitive types on the lower layers. (ii) Because the concentrations of the entire protein components remarkably increase in the lower layers, the agents possessing the higher bound ratios must increase their adsorption more rapidly than those of the lower bound ratios to the lower layers. The former reasoning could be applied to a certain extent to PAS and sulfamethoxypyridazine. However, the latter reasoning could not be applied to any agents employed, though sulfonamide and homosulfamine, whose bound ratios were found to be low by methods (a) and (b), showed a somewhat higher concentration in the upper layers than the other agents.

(10) In contrast to these results, when the adsorption of ^{35}S-DDS and ^{35}S-labeled di-N-acetyl DDS (^{35} S-DADDS) was tested on another serum sample no adsorption of ^{35}S-DDS could be found by method (a), while it was sufficiently detected by method (b). This result again suggests the possibility indicated in (6). ^{35}S-DADDS was found to be almost completely adsorbed on the third pattern.

From the above results, the following two conclusions can be made:

(a) The hypothesis advocated by Tamemasa includes some exceptional cases as indicated in (4), though it furnishes an insight into the importance of long-activity in antileprous agents.

(b) Drugs employed can be classified into several types; the A-affinitive type such as sulfamethoxypyridazine, Ciba 1906, and DADDS, the G-affinitive type such as 1314 TH, the non-adsorptives such as homosulfamine and ethambutol, and their medium type in which DDS, promizol, especially DHDS are included.

From these points of view, the meaning of the drug adsorption in connection with that of the conjugation and demasking in antileprous drugs was discussed together with some problems in the experimental work. (*Adapted from* authors' summary)

Tsutsumi, S., Sakamoto, Y., Gidoh, S. and Nakamura, K. Studies on the mode of action of antileprous drugs. III. On the demasking of several N-conjugates of sulfonamide-type drugs by various strains of microorganisms. La Lepro **39** (1970) 239-240. (In Japanese, English summary)

Demasking in N-conjugates of sulfone drugs and sulfonamides by the incubation with microorganisms was examined. Twenty-one strains of mycobacteria together with four non-mycobacterial strains were employed in the experiment.

Results found were as follows:

1) The deacetylation of mono-Acetyl DDS (MADDS) was generally more ac-

tive in mycobacteria than in the non-mycobacterial strains, especially in such strains as the rapid growing mycobacteria except *M. balnei, M. tuberculosis* H37 Rv, NQ bacilli and non-photochromogens. As for the unclassified mycobacteria, the action in the non-photochromogens was more marked than that in the photo- and scotochromogens employed in the experiment.

2) In spite of active deacetylation in the mycobacterial strains, the action could not be found so markedly or was nearly negative in whole in the N_4-A and N_1-A of the sulfonamides. Tested compounds were: enatoyl MADDS (MEnADDS), monoenantoyl DDS (MEnDDS), and monodiethylamonacetyl MADDS (MEADDS), mono-morpholinoacetyl MADDS (MMADDS), N-acetylates of sulfamethoxypyridazine (respectively abbreviated as N_1ASMP, N_4ASMP, and DASMP), and those of sulfaisoxazole (N_1ASIX, N_4ASIX, and DASIX). Generally, it was found that this strain was apt to demask A more rapidly than En, E, and M from DDS, though the demasking of the latter two was comparatively lower than the former two. The inactivity in the demasking of N_4-A was again noticed in the case of the sulfonamides similarly to (2), and N_1-A suffered severe deacetylation even in the control samples. Therefore, DASMP and DASIX were detected as the corresponding N_4-A even in the controls, as well as the N_1-A were respectively detected as SMP and SIX.

4) Next, the demasking of MADDS by a crude cell-free extract of *M. phlei* (Penso) was examined, and it was found that within three hours under shaking-incubation, a gradual increase of freed DDS could be detected with the lapse of the incubation periods. However, the condensation of the activity failed resulting in the disappearance of the activity during fractionation.

5) Employing H-glucuronate (^{3}H-GNa) and DDSG-^{3}H, which was synthesized from DDS and ^{3}H-GNa, the influence of *E. coli* K-12 on the N-G was examined under the correction of pH during the incubation. Though the intake and the catabolic variation of ^{3}H-GNa freed from DDSG-^{3}H could be noticed, the demasking of N-G by the bacilli could not be elucidated. (*Adapted from* authors' summary)

Vargas, Salvador. Tratamiento actual de la reacción leprosa por talidomida. [Treatment of lepra reaction wtih thalidomide.] Dermatologia (Mexico) **15** (1971) 142-154. (In Spanish, English summary)

The results of treatment with thalidomide of 100 lepromatous patients with lepra reaction are reported. This drug is at the present time the best remedy against lepra reaction. The systemic manifestations disappear within 24 hours, the cutaneous, neural and other symptoms in two to three weeks. In addition there is gain of weight and reduction in sedimentation rate. Side effects are minimal and do not require cessation of treatment. The recommended dosage is 200 mg in the beginning in the majority of cases, and 50 or 25 mg for maintenance. The use of the drug in women needs great caution; it is only indicated when there is no ovulation. It is emphasized that this drug should be employed by dermato-leprologists or by institutions well-prepared for its use.—(*Adapted from* author's summary)

Epidemiology and Prevention

Worsfold, J. W. Leprosy in the North Western Province. Med. J. Zambia 4 (1970) 75-79.

The author gives an account of his work in the field of leprosy in the North Western Province of Zambia from 1947 to 1969. Among the many interesting observations in this paper are: female patients outnumbered males; childhood leprosy was uncommon; 50% of all patients denied any family history; there appeared to be no fear of the disease or fear of speaking about it; the "atypical, unstable group" (presumably borderline) was the largest; and the numbers of new cases have not shown any decline.—W. H. Jopling (*From* Trop. Dis. Bull.)

Torres López, E., Arias Lira, A. S. and Barrera Rios, H. Epidemiológia de la lepra en Tamaulipas. [Epidemiology of leprosy in the State of Tamaulipas, Mexico.] Salud Publica Mex. **12** (1970) 183-193.

The State of Tamaulipas is included in the northeastern area of Mexico endemic for leprosy, which is believed to have developed as a result of infected persons from the regions where the incidence of leprosy is higher joining the regular flow of migrants into the frontier States. In the period 1965-1969, 129 cases of leprosy were registered in residents in the State, representing a morbidity of 10.9 per 100,000 inhabitants; 112 (86.8%) of the affected persons were being regularly examined at one of the health centres. Their geographical distribution is shown on a map; 70% of the patients lived in the frontier zone. The lepromatous type of disease predominated, with 86 cases; the number of cases of other forms of leprosy were: tuberculoid, 32; indeterminate, 9; dimorphous, 2. The skin lesions were generalized in 39 patients, disseminated in 38 and localized in 27. Loss of sensitivity was recorded in 97 patients and neuritis in 62. Lesions of the nasal mucosa were present in 67 patients. The highest incidence occurred in persons over the age of 45 years, followed by those aged between 35 and 44 years. In general, the sex incidence was about 2:1. In a familial study of 31 cases, 12 of the 14 families involved each had 2 affected members, one family had 3 and one had 4. Of the 903 contacts studied, 461 were males.

In 88 of the 129 patients, the source of contagion was not identified; of the remaining 41 the most frequent source of infection was found to be a brother; the next most frequent sources were father, mother and uncle. In 57.4% of the patients the disease had taken from 1 to 9 years to develop; in 19.3% from 10 to 19 years, 14% from 18 to 20 years and more; and in 9.3% it had taken less than one year. With regard to occupation, 42 patients were day-laborers, 37 clerical workers, 31 domestic workers, 8 were traders, 5 school children, and 2 each were workmen, bricklayers, and male dancers. Eighty of them were natives of Tamaulipas; 30 others had been born (and probably also infected) in other Mexican states, and one was a native of Texas, USA; in the remaining 18 patients, birthplace could not be ascertained.

The difficulties inherent in the control of the disease are discussed. As the specific national control scheme has been incorporated in the regular work of the coordinated services of Tamaulipas for only four years, there are probably "a considerable number" of persons with leprosy still undetected, in addition to those not yet under control because of change of residence; and there is probably an increase in previously uncontrolled cases among the large annual migrant population in this frontier zone. However, the majority of persons affected are in districts that have a dermatologist in charge.—F. E. Williams (*From* Trop. Dis. Bull.)

Sehgal, V. N. A study of age at onset of leprosy. Int. J. Derm. **9** (1970) 196-199.

The author investigated 954 patients attending leprosaria in Varanasi district, eastern Uttar Pradesh, India. They were asked the duration of the disease and their age at its onset. Where possible, corroboration was sought from relatives and other observers.

In both lepromatous and non-lepromatous patients (men and women) the peak age of onset was between 20 and 34 years. Onset of leprosy under the age of 10 years is less common than in the two series reported from Chingleput district (Madras State) by Cochrane (*A Practical Textbook of Leprosy*, 1947; and Trop. Dis. Bull. **44** [1947] 1026) and by Mohamed Ali (Trop. Dis. Bull **61** [1964] 1145).

The author's figures were obtained by interrogating patients with established leprosy and are not population surveys.—H. V. Morgan (*From* Trop. Dis. Bull.)

Seal, S. C., Hazra, A. G. and Mukherjee, A. K. Clinico-epidemiological study of leprosy in Calcutta with suggestions for control measures. J. Indian Med. Ass. **57** (1971) 419-425.

This article records a steady state of

leprosy in Calcutta during 1961-1966, the average annual attendance of new cases in three clinics remaining unchanged (about 6,400), thereby suggesting little abatement of the disease. A ten percent sample of 2,552 patients was selected for detailed examination (epidemiological and social) and a five percent sample of another group was visited at home.

The article tabulates distribution of origin of new cases, age groupings, religions, occupations, literacy, housing, and provides notes on sanitation, food and personal habits, and economic status. Most of the data suggest that the general status of patients corresponded to that of the city population, except for a lowered economic position.—G. L. Fite

Quisenberry, W. B. and Levy, S. L. Planning for the modernization of Hawaii's leprosy program. Amer. J. Public Health **61** (1971) 1403-1405.

This unemotional administrative account of the transformation of the leprosy service in Hawaii provides the necessary complementary information to the reports that have appeared in the lay press.

The strict segregation policy instituted in 1865 was based upon the best advice then available (and obtained) in Europe and Asia, and sought to deal with an indigenous leprosy problem made worse by leprosy occurring among immigrant laborers. Molokai became notorious, until redeemed by the dedication of Father Damien and the pen of R. L. Stevenson. Now the old-type Kalaupapa Leprosy Colony at Molokai is to be phased out, despite opposition from the pensioned leprosy patients and some disquiet expressed by neighbors.

The Health Department, conscious of the importance of public relations, has leant heavily on the advice proferred by a Citizens' Committee, and the cooperation of officials and community has produced a sound and acceptable scheme for the control of the diminishing problem of leprosy. Outpatients care is now available, and leprosy has a place in the teaching of medical students.—S. G. Browne (*From* Trop. Dis. Bull.)

Pereira, L. C. and Carvalho, J. O. Statement on leprosy in Parana State, Brazil. Publ. Cent. Estud. Leprol. **11** (1971) 18.

Leprosy in the State of Parana, Brazil, continues very active. The most recent statistics show a predominance of lepromatous cases among the new patients registered, and about half the people with the disease and their contacts are not under control.

The decrease in the registration of new cases during the years 1969 and 1970 does not indicate a retrogression, but rather a decrease in the active search for those who have contracted the disease and a lack of knowledge of early diagnosis by general practitioners.—Authors' Summary.

Other Mycobacterial Diseases

Uganda Buruli Group, The. Epidemiology of *Mycobacterium ulcerans* infection (Buruli ulcer) at Kinyara, Uganda. Trans. Roy. Soc. Trop. Med. Hyg. **65** (1971) 763-775.

The epidemiology of lesions due to *Mycobacterium ulcerans* was studied in an almost closed community of 2500 Rwandan refugees living near the Nile in central Uganda over a period of years during which 220 of them showed the disease. The incidence was greatest in children aged 5-14 years, in those living nearer to Nile, and during the months September to November. In adults it was greater in women than in men. The geographical gradient in incidence was more apparent among women, whereas temporal variation mainly affected the men. Direct contact with the Nile was not necessary for transmission. The disease gave no evidence of spread from person to person and the incidence fell to zero when the people moved to a new locality. The incubation period was usually under three months. Lesions were

usually single; they occurred on any part of the body in children but were largely confined to the limbs in adults. In men, lesions were almost restricted to the lower legs whereas in women the arms were also often affected. Hypotheses of transmission are discussed in relation to these observations.—Authors' Summary

Offer, R. C., Karlson, A. G. and Spittell, J., Jr. Infection caused by *Mycobacterium fortuitum*. Mayo Clin. Proc. **46** (1971) 747-750.

This article reports a case of infection with *M. fortuitum* following a penetrating injury to the lower leg. The injured area remained somewhat swollen but nontender for two months, when a slight serosanguinous drainage appeared. Four months later, following drug medication, the lesion relapsed and when drainage failed to give adequate healing, bacteriologic studies were done which yielded *M. fortuitum* in substantial numbers. The lesion responded well to surgical scraping of a deep subcutaneous cavity and without drug treatment.—G. L. Fite

Kondo, E. and Kanai, K. The lethal effect of long-chain fatty acids on mycobacteria. Jap. J. Med. Sci. Biol. **25** (1972) 1-13.

Lethal effect of long-chain fatty acids on various species of mycobacteria was examined in a buffer of pH 5.6. Oleic, linoleic and myristic acids were most active against all the test strains. Lauric and palmitic acids were also active, but stearic acid was inactive. The unsaturated fatty acids inhibited markedly the activities of the membrane-bound enzymes (acid phosphatase and tetrazolium reductase) of *M. bovis*. The free fatty acid fraction of "lysosomal components" separated from the normal guinea pig lungs consisted mainly of palmitic (30%), oleic (30%) and linoleic acids (15%); consequently the fraction was active in killing mycobacteria. The mycobactericidal activity of free fatty acids was slight at neutral pH and neutralized by a basic protein, protamine. From these observations, discussions were given on the possible conditions required for fatty acids to be involved in the mechanism of resistance to tuberculous infection.—Authors' Summary