

CURRENT LITERATURE

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General and Historical

✓ **Coonaratna, C. de F. W.** Some historical aspects of leprosy in Ceylon during the Dutch period 1658-1796. *Med. Hist.* 15 (1971) 68-78. (*From Trop. Dis. Bull.*)

✓ **Gussow, Z. and Tracy, G. S.** The phenomenon of leprosy stigma in the continental United States. *Leprosy Rev.* 43 (1972) 85-93.

Recent studies on leprosy stigma in the continental United States are presented and critically reviewed. In view of the strong concern about stigma expressed by patients and leprosy workers alike, it is interesting that strong public stigma has not been actually demonstrated scientifically. The evidence is equivocal; leprosy may be stigmatized to some extent, but so are other chronic diseases. The paper advances a social and psychological explanation for some of the more important peculiarities of the phenomenon of leprosy stigma, and concludes that those responsible for the treatment of patients may need to think in terms of alternatives to presumptions of public stigma.—Authors' Abstract

Rotberg, A. The serious Latin-American problems caused by the complex "leprosy: the word, the disease" and an appeal for world cooperation. *Leprosy Rev.* 43 (1972) 96-105.

Two Brazilian Congresses of Hygiene (in 1968 and 1970) agreed that a substitute name for "leprosy" would have psychological advantages in health education, facilitating preventive measures and alleviating the stigma. Accordingly, three state health services, the chairs of dermatology and

many of neurology and preventive medicine of 27 medical schools in Brazil have already adopted the educational formula "hanseniasis, formerly called leprosy," aiming at a gradual elimination of the stigmatizing term leprosy. The complex "leprosy: the word and the disease" is also a problem for other countries of Latin America: education on "leprosy" there was a failure, as recognized by the Guadalajara (Mexico) Seminar of the Pan-American Health Organization (1968). The complex affects also the Gulf States of the United States of America, Portugal, Spain and Italy. Many specialists of Asia, Africa, the Western Pacific and Eastern Europe are already not insisting on conserving in our language a stigmatizing form which in any case does not belong to theirs. Many Europeans and Americans, whose countries do not suffer from an active endemic, are aware of the damage the word is causing to millions in our areas. The solution is relatively easy and consists simply in extending to the root "*lepra*" the international condemnation of the pejorative "*leper*." Usual objections against this extension are presented and rebutted.—Author's Abstract. (Point No. 8, page 104 is factually fallacious.—OKS)

Rovisco Pais, January-March (1971) 1-88.

The January-March issue of 1971, with the help of 47 tables, presents the details of the status of leprosy in Portugal. New cases have been diminishing slowly, and the steady continuing effort to bring leprosy under control continues. At the close of 1970, 582 patients were hospitalized at the Hospital-Colónia, compared with approximately 900 ten years earlier. S. M.

Goncalo's account of the use of B-663, finding it especially valuable in cases of leprosy with apparent sulfone resistance, parallels that observed in other countries. The programs of two courses in leprosy include one of more than three weeks designed for missionaries and hospital personnel in October 1971, and a somewhat shorter course for physicians in September. The titles listed number 31 and 24 respectively. The rest of the issue is given to a wide miscellany of topics of general interest rather than scientific import.—G. L. Fite

Warren, A. G. Are deformities stigmatizing? A surgeon's approach. *Leprosy Rev.* 43 (1972) 74-82.

Disability in leprosy is often equated with the degree of obvious deformity. The patient's psychological approach to his disease influences his disability. The reconstructive surgeon can in many cases assist in rehabilitation by correcting stigmatizing deformities that constitute an obstacle to the patient's acceptance in the community.—Author's Abstract

Clinical Sciences

Basu, A. K., Chatterjee, J. B., Mukherjee, A. and Ghosh, S. Observation on hemostasis in leprosy. *Bull. Calcutta Sch. Trop. Med.* 18 (1970) 33-34.

This short note merely opens the question of whether there may be some disturbance of clotting factors in leprosy; it does not solve it. Five standard clotting tests were performed on 43 patients, none of whom had any bleeding tendency. The celite-activated partial thromboplastin time was abnormal in 24 patients, and the prothrombin time in 15 (both tests were abnormal in 12). In these same patients there were various minor anomalies in the thromboplastin generation test. The euglobulin lysis time was prolonged in eight patients. Three explanations are offered: (1) an effect of hypergammaglobulinemia; (2) disturbance in the synthesis of clotting factors; (3) intravascular fibrin formation. [But these findings will require confirmation and assessment especially in relation to whatever treatment was being given.]—Alan B. Raper (*From Trop. Dis. Bull.*)

Belda, W. Observações sobre a reação de Mitsuda no grupo indeterminado da lepra. *Leprologia* 16 (1971) 67-72. (In Portuguese, English summary)

Considering the predominance of lepromatous patients on the endemicity of leprosy in the State of São Paulo, the author attempted to study the behavior of Mit-

suda's skin reaction in indeterminate leprosy patients.

Within 400 patients submitted to Mitsuda's test, he found 86.5% of nonreactive and weakly reactive results. The author suggests priority care in terms of educational and therapeutic measures for the indeterminate group of patients.—(*Adapted from English summary*)

Cross, A. B. Foot deformities in leprosy. A survey in the Solomon Islands. *Leprosy Rev.* 43 (1972) 45-52.

(1) A survey was carried out on 1,000 lower limbs of leprosy patients. (2) All deformities, except palsies, were more prevalent in patients with lepromatous leprosy. (3) Plantar ulcers occurred in about 26% of feet. (4) There was little difference between the sexes in the incidence of ulceration. (5) The type of terrain had no influence on the incidence of ulceration. (6) The picture of deformity in the Solomon Islands Melanesians is similar to that in Australian Melanesians. (7) Foot care is important in the treatment of leprosy in the Solomon Islands.—Author's Summary

Davison, T. A. and Bernard, J. C. Gingivopatias inflamatorias en lepromatosos. *Leprologia* 17 (1972) 63-67. (In Spanish, English summary)

The most frequent gingival lesions in leprosy were found to be nonspecific, there

being 84% nonspecific gingivitis, 50% gingival amyloidosis, and 14% specific leprous lesions in 50 biopsies from leprosy patients between the ages of 21 and 68 years. The leprosy lesions follow more closely the pattern of visceral leprosy than of skin involvement. Nonspecific lesions were more frequent than specific and chronic lesions more frequent than acute.—(Adapted from English summary)

Muganya, A. W. A review of patients developing reactions at the East Africa Leprosy Research Centre and Alupe Hospital over eight years (1950-1957). *Leprosy Rev.* 43 (1972) 27-29.

In this paper is reviewed the incidence of reactions among both inpatients and outpatients at Alupe Hospital and the East Africa Leprosy Research Centre in the period 1950-57. The number of patients who suffer from reactions form only a small percentage of the whole, but the frequency of reactions among those who do get them is high. The complications that follow reactive phases are emphasized, as well as the duration of individual reactions.—Author's Summary

Rouzaud, P., Graveline, J. and Graveline, M. Les lésions oculaires de la lèpre à Tahiti. [Ocular lesions in leprosy in Tahiti.] *Med. Afr. Noire.* 19 (1972) 45-51. (In French)

The authors summarize their findings of eye lesions found in 43 of 55 patients with leprosy (out of 329 under treatment) who voluntarily submitted to ophthalmological examination. All except one were Polynesians; 33 were men; all patients were over the age of 20 years (the average age being 49); and 39 patients had had leprosy for more than 20 years. The forms of leprosy are given as: lepromatous 33, tuberculoid 12, and indeterminate 8 (which probably means undetermined in this context).

In only 12 patients were the eyes considered to be completely normal. Of the rest, 14 had some degree of madarosis, and 23 paresis or paralysis of one or both facial nerves. Fifty-one of the 110 corneas had

lesions of varying gravity, such as vascularized pannus, fine precipitates in Descemet's membrane, annular degeneration of the limbus. In 37 (out of 110) the iris showed some atrophy, with miliary nodules visible in a further 5 patients. While only one globe had total corneal anesthesia, there was some loss of sensation in 30.

This group of self-selected patients (most of whom had probably consented to be examined because they complained of some eye symptoms) had had the advantages of early diagnosis of leprosy, adequate treatment, and good ophthalmological supervision. While their visual acuity had remained good, the ocular complications listed indicate the importance of eye involvement in leprosy. In this small series, no conclusion can be drawn with regard to the incidence of eye damage in the various forms of leprosy.

The authors advocate recourse to tarsorrhaphy where indicated, and favor local and systemic use of corticosteroids to control acute ocular conditions and to prevent eye damage.—S. G. Browne (Adapted from *Trop. Dis. Bull.*)

Yagi, I., Osawa, Y., Tsutsumi, S., Sakamoto, Y. and Nakamura, K. Studies on the testing methods of the thyroid function and their application to clinical tests in leprosy patients. *La Lepro* 39 (1970) 193-195. (In Japanese, English summary)

Although thyroid function in leprosy has been studied by several clinicians and researchers there had been no agreement as to whether this function is increased or reduced by leprosy, or if the function is at all related to the development of symptoms in leprosy.

The authors examined several methods of testing thyroid function with a view to applying them to the examination of problems in leprosy.

In regard to Hamolsky's method, it was found that the uptake of ^{131}I -triiodothyronine (^{131}I -T₃) by red blood cells should be corrected by measuring the hemoglobin together with the radioactivity, both of which had been eluted from the

red blood cells during and before the routine work. This correction was based on the principle that both the $^{131}\text{I-T}_3$ and the hemoglobin measured by the cyanmeth Hb method were proportional to each other.

Next, a new type of sponge found by Dr. Inagaki of Tokyo Metropolitan Police Hospital was compared with resin sponge. The former consists of acetalized polyvinyl alcohol and is termed PVF. The latter is considered too expensive for tests with a large number of patients in the routine work.

The results were as follows:

(1) When both the size of the sponge and the solution of $^{131}\text{I-T}_3$ were correspondingly diminished, the uptake by the PVF was influenced much more than that by the resin sponge (RS).

(2) The binding of PVF to $^{131}\text{I-T}_3$ was somewhat more fragile than that of RS, because a considerable portion of $^{131}\text{I-T}_3$ taken up was eluted from the PVF, when the former sponge was again contacted with an unlabeled serum.

(3) PVF showed no marked inferiority to RS in the deviation of the uptake values. With a sponge termed new PVF, in which PVF was inserted, a somewhat remarkable deviation of the uptake values was found.

(4) When the same sponge was repeatedly employed in the test, the lowering of the uptake by the PVF was more rapid than that by the RS.

(5) The increase of the uptake by PVF with the addition of phenol or thiourea was similar to that by RS, though in the case of phenol, it was slightly more sensitive in the latter.

(6) The influence of heparin could not be clearly detected in either of the sponges. However, in the case of a double oxalate added to a serum sample in a concentration of 1-36 mg/ml, the uptake by the new PVF increased to a certain extent with the concentration of the anticoagulant, while that by RS was conversely decreased.

(7) The adsorption of $l\text{-}^{14}\text{C}$ -tyrosine or $l\text{-}^{14}\text{C}$ (U)-glutamic acid on both of the sponges was observed, especially in the RS, though the binding was fragile and showed no influence on the uptake.

(8) The proportionality of the uptake values of PVF and RS was certified by employment in tests on leprosy patients and those of hyper- or hypo-thyroidism, as well as on the healthy.

(9) PVF is much less expensive than RS and the serum necessary for one PVF is half the volume of that for one RS.

Based on these results, it was concluded that PVF could be applied to the test of the thyroid function in leprosy patients as well as in the usual routine work.

Therefore, the authors examined the function of various types of 69 leprosy patients in comparison with that of 56 healthy persons and 14 tuberculosis patients by the use of PVF. It was found that the uptake (%) was gradually lowered, while no difference could be found in healthy persons and tuberculosis patients. In addition, the patients showing epithelioid cell reaction (ECR) tended to show a comparatively higher uptake in contrast with those during ENL, though not a large number of cases could be tested. If it can be presumed that the changes in the serum globulin components have no relation to the levels of TBG, the result indicates a tendency in itself of the thyroid function in leprosy patients. This finding coincided well with the results reported by Yagi, in which a remarkable acceleration of metabolism was noticed in leprosy patients showing ECR. ECR is known to be a case in which a fairly abrupt transition from L- to T-type can be seen. Therefore, the result directs our attention to the significance of the thyroid hormones concerned with the healing mechanism of leprosy.

Moreover, it is supposed that peripheral changes can inhibit the oxidoreduction system which links the deiodination to the thyroid hormones. The provocation of ENL by inorganic iodides is a well-known fact in leprosy. Yagi and Osawa have reported on the markedly lowered function in the adrenal cortex of leprosy patients just before the occurrence of ENL. In such a symptom, a lowering of the mechanism to suppress antibody formation and their general adaptation syndrome must be considered. If an inorganic iodide is administered to those patients, it may be likely to irritate

the cells in the periphery tissues, or, in some cases, may bring about an unfavorable influence on the *in vivo* iodine balance (Wolff-Chaïdoff effect).

On the other hand, Dr. Sasaki of this Institute also has reported on severe amyloidosis characterized by amyloid goiter in several leprosy patients, diminishing the resistance to microbial infection or showing resistance to chemotherapy. Also, Dr. K. Kato reported on TBG lowered by the administration of a corticosterone.

Hereupon, considering the complicated factors lying between the thyroid function in leprosy patients and the clinical effects of antileprosy agents, a discussion is developed for the improvement of chemotherapy in leprosy, especially for the significance of the various phenomena in the hormonal organs during long-term clinical trial of antileprosy agents. (*Adapted from authors' summary*)

Yagi, I., Osawa, Y., Tsutsumi, S., Gidoh, Y. and Nakamura, K. A mutual interference between thyroid hormones and antileprosy drugs in the binding to human serum. *La Lepro* 39 (1970) 246-252. (In Japanese, English summary)

In the preceding paper, the possible relation between thyroid function and the healing mechanism of leprosy was discussed.

The authors examined the interference of several antileprosy drugs in the binding of ^{131}I -thyroxine (^{131}I -T₄) to human serum by the use of an *in vitro* experimental method, radiostereoisotopic assay. The drugs employed were di-N-acetyl DDS (DADDS), Ciba 1906, sulfamethoxypyridazine (all of them are representative drugs adsorbed on albumin, A-type as previously reported), 1314 TH (G-type), and DDS.

The ratio of the bound thyroxine in A per G gradually increased with the concentration of 1314 TH added to the serum, which was supposed to be due to the removal of bound ^{131}I -T₄ from G to A by the direct interference in the binding of thyroxine to TBG. While, in the cases of the A-type drugs, the rate was diminished with the concentration of the drugs, or in some cases, diminished and then increased so as to form a valley.

When the concentration of thyroxine increased, the valley was apt to move to the point where the concentration of the drugs was lower than before. This phenomenon can be explained by the concept that the binding site of drugs or even hormones is not restricted by a single component, but by adsorption on several components peculiar to a drug or a hormone. Depending upon this concept, the following explanation is possible. During the diminution of the ratio in A as compared to G, the saturation of TBG by both thyroxine and one such drug may not be reached and the interference by A-type adsorption of the drug to the slight binding of thyroxine to A is merely emphasized.

When TBG is saturated the nonspecific binding of thyroxine to A necessarily increases with the concentration of the drug, thus the valley is formed. In addition, when the concentration of thyroxine or the drug is increased, the mutual interference in TBG may considerably increase, when it is compared with that in A, because the saturation in TBG can be reached more easily than in A and the binding of thyroxine to TBG is well-known to be more stable than to A. Therefore, when the concentration of thyroxine is increased, TBG must be saturated by a lower concentration of the drug, thus the valley moves to the lower concentration of the drug.

Although not so obvious, a similar result in the ratio of prealbumin (PA) per G was detected, as well as in the ratios of A per total and PA per total, in which the total means the total radioactivity of PA, A, and G.

A diuretic, Saltron (4-chloro-1, 3-disulfonamide), also showed a shallow valley. In the case of DDS, this phenomenon was not openly apparent, perhaps due to the comparatively labile binding to human serum, or to a binding type as previously reported.

In conclusion, it can be said that a mutual interference or even a competitive relation between thyroxine and several antileprosy drugs with respect to binding to the protein components of human serum exists, based on an *in vitro* experimental method. (*Adapted from authors' summary*)

Treatment

Bhasin, D. and Antia, N. H. Radical metatarsectomy for intractable plantar ulceration in leprosy. *Leprosy Rev.* **43** (1972) 53-58.

Of the 16 feet operated upon for recurrent ulceration 10 have remained free of plantar ulcers; 5 of these have been ulcer-free for two years or more, and 4 have remained ulcer-free for four years. These figures may not be very impressive in themselves, but they are encouraging when considered in relation to the condition of the feet operated upon.—(Adapted from authors' conclusions)

Ellard, G. A., Gammon, P. T., Helmy, H. S. and Rees, R. J. W. Dapsone acetylation and the treatment of leprosy. *Nature* **239** (1972) 159.

The capacity to acetylate isoniazid was used as a criterion of the acetylation phenotype of patients on the basis of fluorimetric studies of plasma compared to ratios of acetylated to non-acetylated drug excreted.

Of 21 drug resistant patients, 15 were rapid acetylators, and the results suggest that rate of acetylation of dapsone is unlikely to have diagnostic meaning in leprosy.—G. L. Fite

Helmy, H. S., Pearson, J. M. H. and Waters, M. F. R. Treatment of moderately severe *erythema nodosum leprosum* with clofazimine—a controlled trial. *Leprosy Rev.* **42** (1972) 167-177.

In a controlled trial, designed to be double-blind, ten patients suffering from moderately severe *erythema nodosum leprosum* (ENL) were studied for a total of 14 weeks. The trial was subdivided into an initial control period of two weeks followed by a first trial period of four weeks in which either treatment with clofazimine, 300 mg daily, or identical placebo capsules was prescribed, then by a second trial period of four weeks in which the treatment given (clofazimine or placebo) was the reverse of that in the first trial period, and concluded with a final control period of four weeks.

Dapsone was given throughout in a dosage of 100 mg twice weekly.

The results, based on the clinical severity of the ENL, temperature, white blood cell count, total dosage of stibophen prescribed, and total number of paracetamol tablets voluntarily consumed by patients in each of the four periods, showed clearly that the ENL improved during the period in which clofazimine was given. Of the ten patients, eight did not develop any signs of clofazimine toxicity (two developed mild gastrointestinal symptoms for the first few days on clofazimine); they were able to carry on their normal daily activities and did not require any special medical supervision. It is concluded that clofazimine is an effective drug in the treatment of moderately severe ENL, and that it is suitable for domiciliary use.—(Adapted from authors' summary)

Pene, P., Carrie, J., Bourgeade, A. and Boliot, Y. Activités du B663 dans la lèpre lépromateuse. Essai thérapeutique. [Activity of B663 in lepromatous leprosy. Clinical trial.] *Bull. Soc. Path. Exot.* **64** (1971) 407-415. (In French)

The report concerns the treatment of a group of twenty patients with lepromatous leprosy, of whom four had not been treated before, four were bacteriologically negative, and twelve had morphologically degenerate bacilli in skin smears. All were suffering from clinically unstable forms of leprosy in severe reaction, and four were corticosteroid-dependent.

Clofazimine (Lamprene; B663) was given at an initial dose of 100 mg daily for six days a week, increasing to 200 mg daily after a month, and then to 300 mg after a further month. In some patients, the daily dose had to be increased to 400 mg.

The clinical results are described as good, with "remarkable" control of the reactive state. No effect was observed on chronic rhinitis, but cicatrization of plantar ulcers and definite regression of peripheral neuritis were noted. Patients gained weight, and accepted the treatment despite the pigmentation.

The drug was well tolerated by all the patients, except one who complained of some diarrhea when the daily dose was increased to 300 mg. No evidence of toxicity was forthcoming. No instance of acute exacerbation arose during the course of treatment, and the patients are said to have expressed satisfaction with freedom from disabling and distressing reactional episodes.—S. G. Browne (*Adapted from Trop. Dis. Bull.*)

✓ **Rollier, R. and Rollier, M.** Traitement de la lèpre lépromateuse par l'ethionamide. [Treatment of lepromatous leprosy with ethionamide.] *Maroc Med.* 52 (1972) 148-166.

This well-documented account of a trial of ethionamide in leprosy attempts an objective reappraisal of the efficacy of this neglected drug, and thus complements the experimental work of Shepard (*Trop. Dis. Bull.* 68 [1971] abstr. 1013). The trial concerned 102 patients and extended over ten years. Laboratory investigations were adequate, including examinations of skin biopsy specimens and material obtained from the skin (by the slit-smear technic) and from the "nasal mucus."

All the patients, except one, had lepromatous leprosy. The drug was first given at a dose of 1 gm daily; because of side-effects (mainly digestive) the dose was reduced to 0.50 gm daily for an adult, and 0.25 gm for an adolescent. Bacilli disappeared from the nasal mucus and from the skin at a regular rate, and at the end of four years of treatment, in the majority of patients, the Bacillary Index had fallen to zero. (The morphology is not indicated.)

Clinical amelioration was also impressive. Early signs of improvement could be seen after six months of treatment, and after two years 75% of the patients could be classified clinically as "disease arrested." Special attention was paid to lesions of the upper respiratory tract (nasal mucosa, palatal mucosa and the tongue), and to the occurrence of *erythema nodosum leprosum*. Some evidence is given that the latter might on several occasions have been precipitated by the drug when given in the

higher initial dose of 1 gm daily.

The authors conclude that ethionamide is active in leprosy, and that its activity resembles that of dapsone. Given at the dose recommended, it is relatively free from side-effects. Its price militates against its ever being more than a second-line drug.—S. G. Browne (*Adapted from Trop. Dis. Bull.*)

✓ **Schulz, E. J.** Forty-four months' experience in the treatment of leprosy with clofazimine (Lamprene [Geigy]). *Leprosy Rev.* 42 (1972) 178-187.

The author's experience in treating 123 patients with clofazimine is reported. Long-standing corticosteroid-dependent *erythema nodosum leprosum* (ENL) was adequately controlled after an average of seven months of treatment with clofazimine. After an average of 16 months, recurrence of ENL was negligible. Early cases of ENL were easier to control. Patients with neuritis, and those with tuberculoid and borderline reactions and suspected dapsone resistance, also responded favorably to clofazimine. A controlled trial over two and one-half years indicated that while the addition of clofazimine to dapsone in the treatment of patients with lepromatous leprosy did not hasten bacillary clearance, the incidence of reactions was considerably decreased.—(*Adapted from author's abstract*)

✓ **Tarabini-Castellani, G.** El Bactrim en el mal perforante plantar hanseniano. [Bactrim in leprotic perforating plantar ulcers.] *Med. Trop.* 47 (1971) 236-254. (In Spanish, English summary)

This is an account of tests on the action of Bactrim (co-trimoxazole, combination of trimethoprim and sulphamethoxazole) on perforating plantar ulcers in leprosy patients, all of whom lived and worked in a sanatorium village. The study was carried out in two stages. The first was the pilot study, lasting three and a half months consisting of 68 patients divided into two equal groups: A, treated by Bactrim; and B, a control group which received the usual antileprosy treatment. The results for Bac-

trim at a dose of two tablets/day were better than conventional treatment. In a second study, 68 patients were treated with Bactrim and 22 acted as controls, but both groups were comparable in age and chronicity of the lesions to those in the pilot study. Ten of the Bactrim patients were given a loading dose of six tablets/day for 10 days while the control patients were given 0.6 mega units benzylpenicillin (penicillin G) for 20 days, followed by tetracycline one gm/day for 30 days. Some of the Bactrim patients who had not improved after 50 days of treatment were then transferred to the benzylpenicillin-tetracycline (B/T) group, making a cross-over trial. The results were as follows: in 17 patients on Bactrim (10 with tuberculoid and 7 with lepromatous leprosy) the condition was arrested; and in 3 patients on B/T the disease was also arrested. The author points out that the blood levels after Bactrim were on the average as much as 16 mg%, though he states that this is much too high and can only be justified as a loading dose, as the long-term treatment of two tablets/day gave levels of 4.6 mg%. Bactrim was very well tolerated but treatment had to be suspended in four patients and lessened in one. In comment, the author states that doses of Bactrim higher than the two tablets are advised because of their sulpha content and the possible risk of renal damage. Clinically, of 68 patients treated by Bactrim, in 17 the condition was arrested, 30 improved, and 21 remained stationary, while the comparable figures for the B/T treatment were 3, 5 and 14 respectively. In 28 patients treated by Bactrim and controlled radiologically, the condition was arrested in 8, while of 12 treated by B/T, 3 showed arrest. Here the author emphasizes that the presence *in loco* of affected bones is an obstacle to cure and if drug treatment does not produce arrest, excision of the

affected bones is necessary. More patients with tuberculoid leprosy showed arrest than did those with the lepromatous form, but here the author suggests that Bactrim seems to have a special effect on the peripheral nerves and particularly on the neuritis of the peroneal nerves.—W. K. Dunscombe (*Adapted from Trop. Dis. Bull.*)

Wollstein, L. V. Technical remedies for the severely deformed foot in leprosy. *Leprosy Rev.* 43 (1972) 106-113.

In this further paper from the orthopedic workshop the author describes a second walking aid for the leprosy patient with a severely deformed foot, or feet. It can be made from locally available material, costs less than the stay in hospital which would be otherwise necessary, and moreover gives the patient earlier freedom of movement.—*Author's Abstract*

Wollstein, L. V. and Girling, J. Orthopedic appliances for leprosy patients. The fixed ankle brace walker. *Leprosy Rev.* 42 (1971) 158-166.

The authors describe an appliance, the FAB walker, that has been developed to confirm the proposition that if a plaster-of-Paris (PoP) walking cast will heal a plantar ulcer, then an appliance that follows the principles of a PoP cast will prevent reulceration. The weight distribution principles of the PoP cast are described and how these principles are built into the FAB walker. Details are given of the specially shaped sole which allows the patient to walk with a normal gait, even though the ankle is fully immobilized. The step-by-step measurement taking and the procedures of making the walker are described. The types of foot deformities that benefit from the appliance are listed, as well as other advantages and disadvantages.—(*Adapted from Trop. Dis. Bull.*)

Immuno-Pathology

Bernard, J. D. and Vazquez, C. A. Correlacion genito-hepato-adrenal en lepromatosos. *Leprolgia* 17 (1972) 42-51. (In Spanish, English summary)

A study was made of testes, prostates and mammary glands in 48 necropsies on leprosy patients between 1964 and 1971, and these were compared with pathology

of adrenal glands and liver. It was found that testicular atrophy and prostatic adenomas were very frequent in that the testicular lesions were found in 54% of the cases, of which 2% belong to the initial phase, 40% to the interstitial phase, and the remaining 48% to the atrophic phase. Gynecomastia was found in 12.5% which is in accordance with the findings of other reports. Nonlepomatous lesions were most common among the liver lesions, amyloidosis being found in 40% of the cases followed by fatty degeneration and cirrhosis. Lepromatous lesions were third in frequency. The most important adrenal lesions were lipoid depletion and secondary amyloidosis with lepromatous adrenalitis occupying third place.—(Adapted from English summary)

✓ **Bernard, J. D. and Vazquez, C. A.** Alteraciones mieloplasicas adrenales en lepromatosos. *Leprologia* 17 (1972) 55-60. (In Spanish, English summary)

A state of myeloplastic adrenal lesions in lepromatous leprosy indicates that these lesions are found more frequently in leprosy (67%) than in other diseases, such as tuberculosis, cardiac insufficiency, shock, peritonitis, bronchopneumonia, cerebral hemorrhage, peritoneal hemorrhage, and acute pancreatitis. The authors regard myelopoietic foci, agnogenic metaplasia and myelolipomas as representing a common nosologic metaplastic pattern. Other findings such as adrenal lipoid depletion were most frequently observed in shock and similar conditions.—(Adapted from English summary)

✓ **Desikan, K. V. and Iyer, C. G. S.** The distribution of *Mycobacterium leprae* in different structures of the skin. *Leprosy Rev.* 43 (1972) 30-37.

A survey of the various components of the skin for the presence of acid-fast bacilli in 100 cases of lepromatous and borderline leprosy is reported. Bacilli were found to occur in the nerves, macrophages, arrectores pilorum muscles, hair follicles, blood-vessel walls, and the epidermis; in that order. Bacilli were not present in the sweat

and sebaceous glands. The frequent occurrence of *M. leprae* in the endothelial cells lining the blood vessels is an interesting observation and supports the hematogenous spread of the bacilli. The location of bacilli in the hair follicles and epidermis suggests a possible mode of exit of the bacilli through these structures. *M. leprae* appear to remain dormant in the nerves and arrectores pilorum muscles, even in clinically regressed cases, and could possibly account for the relapse of the disease. (Adapted from authors' summary)

Drutz, D. J., Chen, T. S. N. and Lu, Wen-Hsiang. The continuous bacteremia of lepromatous leprosy. *New Eng. J. Med.* 287 (1972) 159-164.

Twenty-five of 32 patients with leprosy and high concentrations of acid-fast bacilli (AFB) in the skin had bacteremia of a magnitude that permitted identification of *Mycobacterium leprae* in smears of peripheral blood. In patients with untreated lepromatous leprosy, AFB are continuously present approximately at a concentration of 10^5 per milliliter of blood and are unassociated with signs or symptoms of septicemia. AFB are found extracellularly as well as in polymorphonuclear leukocytes, monocytes and large circulating histiocytes. Bacilli appear to enter the blood stream through invasion of skin arterioles, capillaries and venules. The presence of bacteremia can be predicted from the appearance of skin and liver biopsies. Bacteremia gradually subsides within four months after therapy with diaminodiphenyl-sulfone is begun, and the disappearance of bacilli from the blood provides an indicator of early response to chemotherapy.—Authors' Abstract

✓ **Farnes, P. and Barker, B. E.** Differentiation of lipid-laden cells in steroid-treated human bone marrow culture. Abstracts: Eighth Annual Mtg. Reticuloendothelial Soc. (1971) 49-50.

In human bone marrow cultures supplemented with small amounts of prednisolone, changes in distribution, morphology,

and histochemistry of stromal cell types occur (Nat. Cancer Inst. Monogr. 26 [1971] 199). In steroid-treated cultures older than two weeks a population of lipid-laden cells had been identified. Human bone marrow was cultured in plastic flasks as monolayers, in NCTC 135 with 20% fetal calf serum, with or without prednisolone (1-4 $\mu\text{g/ml}$). Morphology, alkaline phosphatase activity, and lipid content were studied. Cultures for electron microscopy were fixed with glutaraldehyde and embedded in Epon *in situ*.

In the prednisolone-treated cultures, early migration of explanted fat cells with modulation to elongated form was often observed. Additionally, numerous lipid-laden cells appeared in increasing numbers in pericentral zones around marrow flecks. Lipid-laden cells (LLC) differ from fibroblast-like cells (FLC) in several respects. They have smaller nuclei, larger size, larger and more numerous lipid globules, and do not produce cytoplasmic fibrils. Like FLC, they actively pinocytose and possess alkaline phosphate activity. These features strongly suggest a common progenitor, but differing morphology affected by steroid treatment. Apposition of mitochondria to lipid in LLC and decreasing numbers of LLC in older cultures suggest lipid utilization. It is of interest that lipid-laden "foam" cells were seen in increased numbers in bone marrow of steroid-treated patients. (Adapted from authors' summary)

Gabbiani, Giulio and Majno, Guido. Dupuytren's contracture: Fibroblast contraction? Amer. J. Path. 66 (1972) 131-146.

In six cases of Dupuytren's disease and one of Ledderhose's disease, the nodules of the palmar and plantar aponeurosis were examined by light and electron microscopy. The cells composing these nodules, presumably fibroblasts, showed three significant ultrastructural features: (1) a fibrillar system similar to that of smooth muscle cells; (2) nuclear deformations such as are found in contracted cells, the severest being recognizable by light microscopy (cross-banded nuclei); (3) cell-to-cell and cell-to-stroma attachments. Based

on these data and on recent information about the biology of the fibroblasts, it is suggested that these cells are fibroblasts that have modulated into contractile cells (myofibroblasts), and that their contraction plays a role in the pathogenesis of the contracture observed clinically.—Authors' Summary. (Abstract here included because of occasional inclusion of Dupuytren's contracture in differential diagnosis of leprosy —OKS)

Hoffman, E. The Mikulicz cell: A macrophage. Abstracts: Eighth Annual Mtg. Reticuloendothelial Soc. (1971) 39.

Rhinoscleroma is a granulomatous infection induced by *K. rhinoscleromatis*. The infection is characterized by a tumor-like lesion located mainly in the upper respiratory tract. Microscopically, the lesion is predominantly an infiltration of vacuolated cells, termed "Mikulicz" cells, and plasma cells. Although little is known of the origin, structure, and pathogenesis of the Mikulicz cell, it has been postulated to be either a macrophage or a plasma cell. Studies of six human cases showed that the Mikulicz cell had a foamy cytoplasm, containing on occasion phagocytized bacteria. The nuclei are frequently pyknotic and the cell limits are usually not well-defined. Fluorescent studies with anti-*K. rhinoscleromatis* antibodies denoted a positive reaction in many of these vacuoles. Under the electron microscope this cell was morphologically a macrophage. The numerous vacuoles in this cell were considered to be: (1) heterophagosomes containing bacteria and/or a finely granular material similar to the slime layer of the microorganism. These heterophagosomes were frequently ruptured; (2) autophagosomes with enclosed organelles and other cytoplasmic material; (3) swollen mitochondria. The well-developed Mikulicz cell, on ultrastructural basis, is clearly a degenerated macrophage. The plasma cells which were present were readily distinguished from this cell. The pronounced vacuolization of the Mikulicz cell appears to be the result of endocytosis of the slime layer of *K. rhinoscleromatis*. This mucopolysaccharide probably pro-

duces an osmotic imbalance within the phagosome leading to the ultimate destruction of the macrophage. (*Adapted from author's summary. Abstract included here because of occasional inclusion of Rhinoscleroma in differential diagnosis of leprosy. OKS*)

Jopling, W. H. and Mehta, H. D. A case of leprous nodular interstitial myositis. *Leprosy Rev.* 43 (1972) 39-43.

Although the presence of lepromatous granulomata in skeletal muscles is by now well-known, these usually produce no symptoms or clinical signs. This is a report of a patient who developed firm and well-defined nodules within the muscles of the calves and thighs while under treatment with dapsone. The histological findings are described.—Authors' Summary

Kenefick, J. S. Neural leprosy. *Brit. J. Clin. Practice* 26 (1972) 227-228.

This report from the Royal Free Hospital, London, is of an Indian girl aged 14 years in whom, apart from a painless swelling 8 inches long and $\frac{1}{4}$ inch wide on the radial border of her right forearm which had been present for three years, there were no other manifestations of the disease. Her only complaint was that her thumb "felt that it did not belong to her." There was slight hypoesthesia in the cutaneous distribution of the terminal branch of the radial nerve. At operation, the thickened nerve contained tough, tenacious yellow material which dissected out. Histological examination showed "the typical appearance of tuberculoid leprosy." The lepromin test was strongly positive—F. I. C. Apte (*Adapted from Trop. Dis. Bull.*)

Masubuchi, Shigeo. Enzyme histochemical property of human lymph nodes: its immunological interpretation. *Acta Path.* 22 (1972) 107-130.

Lymph nodes surgically removed from patients with various diseases were histochemically investigated. Enzymes examined were alkaline phosphatase (AIP), acid phosphatase (AcP), β -glucuronidase

(β -G) and N-acetyl- β -glucosaminidase (NGa). On the other hand, adult albino rabbits and Wistar rats were immunized by horseradish peroxidase (HrP) mixed with Freund's complete adjuvant and the localizations of these enzymes, antigen HrP and its antibody in their regional lymph nodes at various stages of the immunization were investigated.

AIP in the human lymph nodes was mainly localized in the reticulum cells of the paracortical areas in a characteristic network pattern. This pattern was most prominent in the lymph nodes of SLE patients with elevated serum γ -globulin. In the regional lymph nodes of the albino rabbits immunized with HrP, both AIP and anti-HrP antibody were localized along the processes of reticulum cells in the paracortical areas showing a similar network pattern.

The β -G activity in the human lymph nodes was very intense and particularly in plasma cells and lymphocytes in the medullary cords and paracortical areas. The proliferation of these β -G positive plasma cells and lymphocytes in the medullary cords and paracortical areas was most remarkable in SLE patients. In addition, the β -G activity in these cell types responded most readily and steadily to the antigenic stimulation of HrP to both albino rabbits and Wistar rats.—Author's Summary

McKeever, P. E., Douglas, J. S. and Gee, B. L. Action of theophylline and propranolol on phagocytosing alveolar macrophages (AM). Abstracts: Eighth Annual Mtg. Reticuloendothelial Soc. (1971) 10-11.

Several studies have implicated cyclic AMP in phagocytic mechanisms in leukocytes and peritoneal macrophages. Further, there is evidence for β -adrenergic receptors in human leukocytes. We therefore examined the effect of pharmacological agents presumed to act via β -receptors and cyclic AMP. Rabbit AM, obtained by pulmonary lavage, were studied *in vitro* in KRP-serum (12%)-glucose (5.5 mM) medium with heat-killed *Staphylococcus epidermidis* as the test particle. Results were: (1)

propranolol (1 mM) inhibited both glucose oxidation and particle entry; (2) theophylline (3 mM) inhibited glucose oxidation without effects on particle entry; (3) dibutyl cyclic AMP, PGE₁ and PGE₂, had minor inhibitory effects of glucose oxidation, while epinephrine and isoproterenol were without effects; (4) the effect of propranolol could not be reversed by isoproterenol (1-0.1 mM), nor by increasing Ca²⁺.

While no certain conclusions can be drawn without measurements of cellular cyclic AMP levels, these data suggest that: (1) cyclic AMP modifies glucose, metabolism of alveolar macrophages; and (2) the action of propranolol is probably independent of β -blockade.—(*Adapted from authors' summary*)

✓ **Moretti, G. and Bertamino, R.** Il monotest nella lebbra lepromatosa. [The monotest in lepromatous leprosy.] *G. Ital. Derm.* **46** (1971) 53-54.

The heterophile antibodies in lepromatous leprosy, as observed using the Monotest, were increased in 6 of 20 patients in a state of reaction, in 2 of 8 patients with purely cutaneous reactivity, and in 3 of 22 clinically stable.—G. L. Fite

Paik, Y. K., Kimura, J., Yanagihara, E., Ochiai, H., Tam, R., Murakoka, M. and Hokama, Y. Inhibition of the phytohemagglutinin stimulation of leukocytes by C-reactive protein. Abstracts: Eighth Annual Mtg. Reticuloendothelial Soc. (1971) 30-31.

It had been shown recently (Sample *et al*, *J. Nat. Cancer Inst.* **46** [1971] 1291) that serum factor(s) in individuals with various cancers inhibited the phytohemagglutinin (PHA) stimulation of lymphocytes. It was, therefore, of interest to us to examine the effects of both CRP and BGS on the ³H-thymidine incorporation in the presence and absence of PHA in the mixed leukocyte (MLC) and leukocyte (LC) cultures.

Addition of 1.0-4.0 μ g N CRP into MLC demonstrated no inhibition at lower levels to 46.0% inhibition at the higher levels of

CRP on ³H-thymidine incorporation in the trichloroacetic acid-precipitable fraction. Four micrograms N CRP added to LC showed maximum inhibition of 34.0% of ³H-thymidine incorporation. Various concentrations of PHA alone added to LC showed ³H-thymidine incorporation of 1.32-5.00 times those of the control LC values. Addition of purified CRP in amounts of 2.0-4.0 μ g N LC in the presence of PHA resulted in the suppression of ³H-thymidine incorporation. These ranged from 19.7% to 65.5% suppression from the lower to higher concentrations of CRP, respectively. It is suggested that the suppression of ³H-thymidine incorporation of lymphocytes attributed to serum factor(s) noted in patients with cancer may, in part, be due to CRP. Furthermore, it is suggested that CRP inhibits by binding to the phospholipids (containing phosphorylcholine residues) on the lymphocyte surface and thereby through steric inhibition preventing internalization of PHA necessary for lymphocytes blastogenesis.

BGS added to MLC and LC at 2.0 mg/0.5 ml culture medium appeared to stimulate ³H-thymidine incorporation. Addition of CRP to the same culture mixture partially reversed the stimulatory effect of BGS.—(*Adapted from authors' summary*)

Shabalova, N. N. The influence of neonatal thymectomy in rats on formation of inflammatory reaction and barrier function of the lymphoid tissue. *J. Microbiol. Epidemiol. Immunobiol. N-1* (1972) 39-42. (In Russian, English summary)

The author has examined the barrier function of the lymphoid tissue, and the rate of formation of the cellular component of inflammatory reaction in neonatally thymectomized, and sham-operated rats. Neonatal thymectomy was found to disturb the barrier function of the lymphoid organs to *S. paratyphi B*, contributing to the generalization of the infectious process.

The intensity of formation of a cellular component of the local inflammatory reaction in thymectomized rats proved to be much less, in comparison with the sham-operated animals.—(*Adapted from authors' summary*)

Shepard, C. C. and Karat, A. B. A. Infectivity of leprosy bacilli from bone marrow and liver of patients with lepromatous leprosy. *Leprosy Rev.* 43 (1972) 22-26.

Specimens of bone marrow or liver were collected from patients with lepromatous leprosy at the same time that skin biopsy specimens were removed. The viability of the bacilli was measured by inoculation into mice. There were 20 bone marrow specimens, and infectivity for mice was present in all of the 16 that came from patients whose skin specimens contained bacilli infective for mice. The incubation period was shorter with skin specimens in ten cases. It did not differ by more than two months except in three cases, and in all three it was shorter with skin specimens. Thus, although viable *M. leprae* were present in marrow, the evidence suggests that in most instances the proportion of viable bacilli was somewhat less than in skin.

Five liver specimens were studied similarly. The bacillary viability in these specimens appeared also to be somewhat less than in the skin specimens.

These results are consistent with the hypothesis that the primary site of bacillary multiplication in lepromatous leprosy is in the skin and nasal mucosa, and that the bacilli enter the bloodstream from these sites, to be removed by phagocytes of the bone marrow and liver and other members of the reticulo-endothelial system.—(Adapted from authors' summary)

Terencio de las Aguas, J. and Hierro, Julio del. El antígeno Australia en la lepra. *Rev. Leprol.* 18 (1971) 347-361. (In Spanish, English summary)

The Australian Au antigen was investigated in 211 leprosy cases, 182 inpatients and 29 outpatients. The Australian Au antigen was positive in 11.37% of patients. The positivity was higher in the inpatients (12.8%) than in the outpatients (6.89%).

Regarding the clinical form of the disease, positivity was higher in lepromatous (12.4%) than in tuberculoid cases (5%).

Regarding sex, the frequency of positivity was very similar in males as well as females (12.50% and 11.29% among inpa-

tients and 6.66% and 7.14% in outpatients respectively).

Investigation of healthy people in the surrounding area presented a positivity of 2.38%.

Consideration is given to the frequency of virus-hepatitis in leprosia and to genetic and immunologic factors in relation to positivity significance of the Australian Au antigen in leprosy.—(Adapted from English summary)

Weislow, O., McAlak, R., Sajid, M. A., Mills, L. and Friedman, H. Migration *in vitro* of spleen, thymus and bone marrow cells: effects of irradiation and drug treatment. Abstracts: Eighth Annual Mtg. Reticulo-endothelial Soc. (1971) 27-28.

Earlier studies from this laboratory, as well as more recent studies by others, have established that lymphoid cells other than typical macrophages readily migrate on glass surfaces *in vitro*. Rapid migration occurs from capillary tube culture containing spleen, lymph node, thymus or bone marrow cells. A variety of physical and biologic factors other than antigen-antibody reactions can influence such migration. In the present study the effect of irradiation and/or immunosuppressive drugs on migration were studied in detail. In addition, the effect of irradiation or drug treatment on the migration properties of lymphoid cells transferred to treated recipients was determined. Both irradiation and cyclophosphamide interfered with the "normal" migration activity of spleen and thymus cells. Cyclophosphamide or busulfan alone had little effect on bone marrow cell migration. However, cyclophosphamide plus busulfan resulted in marked inhibition of marrow cell migration. The duration of migration inhibition was greatest in irradiated mice, since recovery occurred more rapidly in drug-treated animals. Transfer of normal syngeneic spleen, thymus, or bone marrow cells to irradiated or drug-treated recipients partially restored migration activity of homologous tissue. Lymphoid cells from recipients of spleen cell transplants showed the best *in vitro* migration; thymus or bone marrow cells, either alone or in

combination, were much less effective. These findings further emphasize that irradiation and immunosuppressive drugs may have different effects on lymphoid cell properties, including *in vitro* migration.—Authors' Summary

Yang, H. Y., Su, W. P. and Skinsnes, O. K. Effect of neonatal thymectomy on macrophage response to *Mycobacterium lepraemurium* infection. Abstracts: Eighth Annual Mtg. Reticuloendothelial Soc. (1971) 19-20.

Observation of immune enhancement of macrophage function and suppressed growth of intracellular mycobacteria led us to believe that host resistance to mycobacterial infection probably depends largely upon the ability of phagocytes to dispose the infective organisms. Altered resistance to mycobacterial infection on infants born with thymic abnormality was attributed to defective cell-mediated immunity, mediated by small lymphocytes. In such instances, the participation of macrophages was not fully evaluated. The present study was intended to explore directly the role of macrophages of neonatally thymectomized mice in combating an obligate intracellular pathogen.

Newborn Swiss albino mice were thymectomized and infected with *M. lepraemurium* by intraperitoneal route within 24 hours after birth. Control mice were sham-thymectomized and similarly infected. Animals were killed at four, seven, and ten weeks of age and the tissue sections were examined. This preliminary study demonstrated that neonatal thymectomy increased the animal's susceptibility to infection. Thymectomized mice showed an early dissemination of infection. Bacterial count of tissue (spleen and liver) homogenate at the end of ten weeks revealed ten times as many bacilli in the thymectomized group as in the controls.

Quantitative study of the peritoneal cells disclosed that within the first five days of infection the macrophage responses were similarly inadequate in both groups. Thereafter, however, the total number of peritoneal cells and the number of large macrophages increased rapidly in the controls but not in the thymectomized group. Four weeks after infection, lepromas developed on serosal surface of peritoneal cavity. Study of these lepromas on omental spread by autoradiography and histochemical technic further revealed impairment of macrophage response of the thymectomized mice.—Authors' Summary

Microbiology

Draper, P. The walls of *Mycobacterium lepraemurium*: chemistry and ultrastructure. J. Gen. Microbiol. 69 (1971) 313-324.

Methods are described for isolating the walls of the intracellular parasite *Mycobacterium lepraemurium* in good yields. The walls were treated with solvents and reagents and their effects were followed by chemical analyses and electron microscopy. The walls resemble those of other mycobacteria, consisting of mucopeptide, arabinogalactan and ester-linked lipid. The information available so far offers no explanation of the bacterium's outstanding resistance to destruction by phagocytic cells. (From Trop. Dis. Bull.)

Jenkins, P. A., Marks, J. and Schaefer, W. B. Thin-layer chromatography of mycobacterial lipids as an aid to classification: The scotochromogenic mycobacteria, including *Mycobacterium scrofulaceum*, *M. xenopi*, *M. aquae*, *M. gordonae*, *M. flavescens*. Tubercle 53 (1972) 118-127.

Examination of the lipids of scotochromogenic mycobacteria by thin-layer chromatography has led to the following conclusions.

1. Organisms which have been classified as *M. scrofulaceum* should be regarded as pigmented types of *M. intracellulare*.
2. The specific name *M. aquae* should be revived and the species defined using

ATCC 19277 as the type strain. Members may be identified by their characteristic lipid structure which is related to that of *M. kansasii*.

3. *M. gordonae* may be readily identified by its lipid structure which indicates a relationship to the nonchromogens previously described as 'provisional species 2.'

4. A new entity apparently of clinical significance is described with cultural resemblances to *M. gordonae* but with a distinctive lipid structure. It has been designated 'provisional species 3.'

5. Eugonic scotochromogens classified as *M. flavescens* exhibit a variety of lipid structures. It is considered, however, that subdivision of the species would serve no useful purpose.—Authors' Summary

Levy, Louis. Prolongation of the lag phase of *Mycobacterium leprae* by dapsone. Proc. Soc. Exp. Biol. Med. **139** (1972) 263-266.

The results of this study support the inference drawn from an earlier experiment that DDS acts on the organisms to prolong their lag phase of multiplication. The time required for multiplication of *M. leprae* to the plateau level of 10^6 organisms per foot pad was shown to be prolonged for early passages from untreated mice; later, the time-to-plateau had decreased for the passages of organisms from treated animals relative to that for the passages from untreated animals. If the differences between the time-to-plateau for control organisms and those for organisms harvested from treated mice may be assumed to represent the prolongation of the lag phase of *M. leprae* by DDS, the lag phase appears to have been prolonged only for the first six to seven weeks after stopping drug administration, subsequently returning to that characteristic of the organisms recovered from untreated animals.—Author's Summary

Prabhakaran, K., Harris, E. B. and Kirchheimer, W. F. The nature of the phenolase enzyme in *Mycobacterium leprae*: structure-activity relationships of substrates and comparison with other copper

proteins and enzymes. Microbios **5** (1972) 273-281.

The phenolase of *Mycobacterium leprae* which oxidized L- and D-DOPA to quinone also metabolized a variety of phenolic compounds with substitutions in the side chain and in the benzene ring. This was different from the condition in mammalian tyrosinase, which was relatively specific L-DOPA. For binding of the substrate molecule to the enzyme in the bacillus, the hydroxyl group in position three of the benzene ring seemed to be essential. At high substrate concentrations, the velocity of mammalian tyrosinase rapidly fell; no inhibitory effect was observed in *M. leprae*, under similar experimental conditions. Comparison with other copper proteins and with peroxidase and catalase indicated that the phenolase of *M. leprae* is probably a true *o*-diphenoloxidase (E.C. 1.10.3.1). Compounds which inhibited the phenoloxidase of the bacillus also suppressed its multiplication in mice, suggesting that phenolase might be of metabolic significance to the organism.—Authors' Summary

Ribichini, J. J. and Giménez, M. M. Estudio histoquímico de los mucopolisacáridos del *Mycobacterium leprae*. [Histochemical study on the mucopolysaccharides of *Mycobacterium leprae*.] Leprologia **15** (1970) 71-73. (In Spanish)

Skin biopsy specimens taken from lepromatous leprosy cases were divided into two groups: (a) nontreated cases, and (b) treated cases. These specimens were analyzed light microscopically after the procedure with the combined method of PAS and alcian blue stains.

In nontreated cases (a) bacillary-formed bacilli usually showed PAS negativity whereas the granular formed ones did positive results. In the cases of longstanding therapy (b) the typical bacillary-formed bacilli were less in numbers. Centrally the granular-formed ones showed accumulations of PAS positive substance. These bacilli seemed to lose their normal structures, consequently they were not viable. No alcian blue positiveness of bacilli was

observed on the bacilli in both groups above. Consequently *Mycobacterium leprae* contained no acid mucopolysaccharides.

The authors were convinced that the clinical improvements of lepromatous leprosy cases were parallel to the increased PAS positiveness of *Mycobacterium leprae* on skin biopsy specimens.—(Abstracted from Leprologia)

Wiygul, W. C. and Rightsel, W. A. Growth of *Mycobacterium lepraemurium* in diffusion chambers containing human embryonic skin cells and in cell-free chambers. J. Gen. Microbiol. 68 (1971) 375-380.

The diffusion chambers and their implantation and removal have been described previously (Rightsel and Wiygul, Trop. Dis. Bull. 68 [1971] abstr. 1987). The chambers were implanted in mice or guinea pigs or in cultures of human embryonic skin cells, and were inoculated with

9.13×10^5 *Mycobacterium lepraemurium*. Half of the chambers were also inoculated with 10^6 human embryonic skin cells. Killed *M. lepraemurium* were inoculated as controls. Four of each type of chamber were harvested after 20, 30, 40 and 50 days of incubation. The yields of *M. lepraemurium* from chambers with embryonic skin cells were approximately twice the yields from cell-free chambers. The maximum increase was 28.8-fold in the chambers maintained in mice, reaching 1.5×10^7 bacilli. After 50 days the yield from chambers with cells in guinea pigs was 1×10^7 bacilli, and from chambers in Petri plates with cells was 7×10^6 bacilli. Thus the authors have shown that *M. lepraemurium* can multiply in a cell-free environment, and they conclude that the peritoneal fluid of the mouse apparently contains "more of the required growth factors than guinea pig fluids."—C. S. Goodwin (Adapted from Trop. Dis. Bull.)

Experimental Infection

Esiri, M. M., Weddell, A. G. M. and Rees, R. J. W. Infection of murine striated muscle with *Mycobacterium leprae*: A study by light and electron microscopy. J. Path. 106 (1972) 73-80.

Thirty-seven CBA mice, 22 of which had had their immunological capacity reduced by thymectomy and total-body irradiation, were infected locally in the foot pad or by the intravenous route with *M. leprae*. Striated muscle from the foot pad and nose was examined by light and electron microscopy to determine the distribution of bacilli and pathologic changes produced during a two year period after infection.

In the normal mice, after an initial short-lived inflammatory response the only remaining bacilli were found to have colonized striated muscle, where they multiplied very slowly and produced after several months a low-grade inflammation with slight patchy muscle fiber damage. In thymectomized irradiated animals there was greater multiplication in muscle and more

widespread muscle damage and inflammation. Pathologic changes seen by electron microscopy in the infected muscle are described; they are considered to be a direct consequence of intramuscular infection by *M. leprae* and not secondary to nerve damage.

It is suggested that, at least in mice, striated muscle forms an environment well-suited to the growth and multiplication of *M. leprae*.—(Adapted from authors' summary)

Ivanova, N. A. Some aspects of the pathogenesis and pathomorphology of leprosy. Bull. WHO 46 (1972) 515-521.

This article reports generalized infection in mice inoculated in the foot pads. A lengthy "incubation" period of four months or more elapsed, and approximately two years were required for infection to become general. The excellent illustration exhibits a varied tissue reaction, tuberculoid with bacilli in some lesions, perhaps never as

frankly lepromatous as in human lepromas. Lesions of nerves of limited degree are described in these animals.—G. L. Fite

Sushida, K. and Hirano, N. Inoculation test with human leprous material into the testis of mice previously injected with sodium iodine (^{131}I). *La Lepro* 40 (1971) 184-193. (In Japanese, English summary)

The thyroid tissue becomes destroyed in mice previously injected with sodium iodine (^{131}I); this destruction causes the body temperature to fall. Leprous bacilli were injected into the above-treated mice and the multiplication process of the bacilli was examined.

The mice were injected subcutaneously in the back with sodium iodine, 50 μC per each animal. Approximately a month later human leprous material was injected. At this juncture the average body temperature of the mice fell about 1°C in contrast with the average body temperature of the control animals. It is generally recognized that the lowering of body temperature is related to the destruction of thyroid tissue. Into the testes of these mice, human leprous material designated as LL11, 21, 22, and 23, was injected. This leprous material was emulsified and diluted. The number of bacilli injected were from 15,000 to 40,000 per mouse. In this dilution, of course, small bacterial groups were found. The harvests of acid-fast bacilli in the testes were examined microscopically every month for 16 months after the injection.

Results. Up to six and eight months after injection of the leprous material into the testes of mice injected with sodium iodine and the control animals (those having no sodium iodine), acid-fast bacilli were found scattered or gathered extracellularly (+s), small groups of bacilli were also sometimes found. Up to 10 and 16 months after injection of treated mice, the cells with acid-fast bacilli were found to be abundant, forming the so-called globi (+G), which were often found in the capsules of the testis by histologic examination. The round forms of globi were not found in this experiment, but spindle forms of globi were observed. The acid-fast bacilli in the globi changed into segmented forms 16 months later.

From these results, it can be inferred that the natural resistance of mice injected with sodium iodine is less than in the control mice. It was considered that resistance of the reticuloendothelial system (RES) had been diminished by the sodium iodine injection.—(Adapted from authors' summary)

Sushida, K., Hirano, N. and Nakano, H. Clearance test of the blood of the mouse injected with sodium iodine (^{131}I). *La Lepro* 40 (71) 194-198. (In Japanese, English summary)

The effect of sodium iodine on the innate immunity of mice was studied by the clearance of the blood by the reticuloendothelial system (RES). Mice were injected with sodium iodine (injection dose was 50 μC per animal). After the injection, three intervals of time were set up. These were (a) a three day interval after injection, (b) a two week interval after injection, and (c) a five week interval after injection. Nine mice were used for each of the above three time intervals, a total of 27 animals.

Next, all nine mice of the three day interval were injected intravenously with 1 mg of *E. coli* suspended in 0.1 ml of NaCl solution. The *E. coli* had been cultivated for 24 hours in an incubator. Then three mice were selected from the nine, immediately after the injection of *E. coli*, and blood was taken from the heart by means of a syringe containing sodium citrate. A group of three other mice were similarly treated at a 30 minute interval after the injection of *E. coli*, and the remaining three mice at a 60 minute interval after the injection.

The amount of blood sample taken by the syringe was 0.5 ml. Next, the liver, spleen and testes of each mouse were taken and emulsified. The concentration of these emulsions was adjusted to 100 mg/ml, and the same organ emulsions of the three mice in each group were pooled. Then, samples of blood, liver, spleen and testes were diluted with NaCl solution, poured into Petri dishes together with melted agar and cultivated for 48 hours in an incubator.

The bacterial colonies on the plates were

counted and the average number of bacteria in 1 ml of blood was compared with that in the control animal. Similar procedures were repeated on the nine mice in the two week and five week intervals.

The results indicated that in the nine mice injected with 50 μ c of sodium iodine and injected intravenously three days later with *E. coli*, the bacilli in the 30 minute animals were diminished and much more so in the 60 minute animals. The clearance ability of sodium iodine injected mice was about the same as in the control mice. In the nine mice which were injected with sodium iodine and injected intravenously two weeks later with *E. coli*, the 30 minute animals showed a decrease of bacilli in the blood stream, and the 60 minute animals

showed slightly more diminution. The untreated control mice showed a greater diminution of the bacilli (*E. coli*) than the treated mice. When the mice were injected with *E. coli* five weeks after the sodium iodine injection, the 30 and 60 minute animals showed abundant numbers of bacilli in the blood stream in contrast to the diminution of the clearance ability in the control mice. Human leprous material was injected into the testes of these animals. The average body temperature during this week indicated a fall of about 1°C. The clearance ability of the liver, spleen and testes of the mice was tested, but it was not significant as shown by figures of the blood stream.—(Adapted from authors' summary)

Epidemiology and Prevention

✓ **Mali, I. B.** Leprosy in Nepalese children. *J. Nepal Med. Ass.* 9 (1971) 207-208.

A survey of schoolchildren in Kathmandu Valley, ages 6 to 14, yielded an incidence of 0.8%, of which more than 90% were nonlepromatous. No deformities were seen. Another study at Re Khoknen Leprosarium, compromised by closer contacts of the children with leprositic adults, showed 5% suffer from the disease. This is regarded as a surprisingly low incidence. No cases under five years of age were found.—G. L. Fite.

✓ **Ojha, K. S., Mathur, G. M. and Sharma, Rashewar.** Some factors influencing the age of onset of leprosy. *Indian J. Med. Sci.* 26 (1972) 160-164.

This study resulted from an inquiry of epidemiological nature of patients in Jaipur, and included 151 clinically known cases and 19 additional cases detected during the course of the study. Thirty percent of the cases were lepromatous. Ages of onset were 30 years in those with definite history of contact with patients, slightly more than 36 years in those without known contact, and 16 years in the 19 cases detect-

ed on home visits. The authors think that these differences stem largely from failure to recognize the disease in its early phases.—G. L. Fite

✓ **Rao, P. S. S., Karat, A. B. A., Kaliaperumal, V. G. and Karat, S.** Incidence of leprosy in Gudiyatham Taluk, South India. *Indian J. Med. Res.* 60 (1972) 97-105.

Estimates of incidence of leprosy are presented for the rural areas of Gudiyatham Taluk based on prospective studies made during the years 1964-1970. During 1967-1969, excluding Gudiyatham town, 1,386 new patients were registered in the taluk of whom 637 had reported the duration of disease as one year or less at the time of registration. Considering all new registrations (Group A), the lepromatous type of cases form 12% and tuberculoid 58%. The male-female ratio was 5:4 and 22% of cases were below ten years of age. Among 637 cases whose reported duration of disease was one year or less at the time of registration (Group B), 6% were of lepromatous type and 62% were of tuberculoid type. The male-female ratio was 1:1 and 32% were below ten years of age. The

crude registered incidence rate varied between 1.1 and 1.5 per 1000 per year. Considering only those new cases with reported duration of disease one year or less at the time of registration, the incidence rate varied between 0.5 and 0.6 per 1000 population per year. The age specific incidence rate was highest for the age group 10-14 years. The incidence rate was significantly higher for the male in Group A but not in Group B. The tuberculoid incidence rate was relatively highest being at least four times more than that of the other types. A direct correlation between the incidence rate and existence of familial cases of leprosy was observed. The total prevalence ratio as well as the lepromatous prevalence were positively correlated with the incidence rate. The implication of the findings are discussed and lines of future analyses indicated.—(*Adapted from authors' abstract*)

Rev. Venez. Sanid. Asist. Soc. 36 (1971) 1-34.

This issue consists of two parts. The first offers a resume of leprosy in Venezuela. The second (20 pages) provides a total bibliography of Venezuelan publications.

A brief introductory review of leprosy by Castellazzi, Arango and Enrique Rasi is followed by a detailed account of the attack on leprosy by Venezuela's "Division de Dermatologic Sanitaria," headed by Convit, by Albernoz M., Arvelo, Rasi, Castellazzi, and Rivas. This is followed by an account of problems of hand deformity in leprosy by Arvelo. Discussion of the public health and occupational problems of leprosy (Albernoz M.) is followed by consideration of problems of rehabilitation by Arvelo, Camevari Pietir, and Convit. Additional articles discuss physiotherapy of the hand, psychological aspects, and other problems in leprosy.—G. L. Fite

Rosemberg, J. and Rocha Passos Filho, M. C. BCG oral e reação lepromínica. [Oral BCG and the lepromin reaction.] *Rev. Brazil. Leprol.* 37 (1970) 51-60. (In Portuguese, English summary)

This is a review of previous work by Rosemberg and by other workers. For the details the original must be consulted.

The authors conclude that BCG vaccination exerts an indisputable effect upon the lepromin reaction, achieving in certain circumstances a conversion in 100% of cases. There is no difference whether the vaccination is administered orally or parenterally. Ingestion of BCG orally: (a) transforms lepromin-negative persons into Mitsuda-positive ones; (b) produces positive lepromin reactions in persons who were negative when tested years earlier; and (c) can intensify lepromin reactions which are already positive. Oral vaccination with BCG shows clearly that positive Mitsuda reactions are produced independently of allergy; the reactions occur in the same form whether tuberculin allergy is previously present or absent. There is dissociation between allergy to tuberculin and the reaction to lepromin, the two phenomena being independent of one another. Immunity to leprosy (as measured by the Mitsuda reaction) can be created without the occurrence of sensitization to tuberculin. All the observations under review strongly suggest that BCG, whether given orally or parenterally, exercises a specific protection against leprosy.—F. Hawking (*From Trop. Dis. Bull.*)

Schaller, K. F. Die Lepra in Athiopien. [Leprosy in Ethiopia.] *Tropenmed. Parasit.* 23 (1972) 98-109.

The overall 10/1000 incidence of leprosy in Ethiopia is lower than that of some central and more southern areas of Africa, yet high, with more than 66,000 registered patients. Highest rates were found in the central high plateau areas. Age distributions and epidemiologic ratios are similar to those of areas in which lepromatous forms are prominent. Systematic attack on the disease was inaugurated in 1954, and continues to be therapeutically aimed at the "outpatient," a single German Leprosy Relief Association Hospital being available at Bisidimo. Disabilities present tremendous problems and have been related to "too little and too late" treatment. From a reading of this interesting article, one concludes that much work remains to be done.—G. L. Fite

Other Mycobacterial Diseases

Dannenbergh, A. M., Ando, M. and Shima, K. Macrophage (MN) turnover and their rate of developing cellular immunity in tuberculous lesions. Abstracts: Eighth Annual Mtg. Reticuloendothelial Soc. (1971) 19.

A single pulse of tritiated thymidine (H^3T) was injected intravenously into control and previously vaccinated rabbits 1 day before or 6, 14, or 27 days after dermal BCG lesions were begun. Periodic biopsy specimens were taken, frozen and sectioned in a cryostat. They were stained for the lysosomal, enzyme β -galactosidase, autoradiographed, stained for acid-fast bacilli, and counterstained with hematoxylin. The local rates of MN turnover and division were determined from the H^3T -labeling data. Their rate of developing cellular immunity was determined from the intensity of the β -galactosidase staining and the disappearance of bacilli.

Our results show that MN continually entered and continually were killed in tuberculous lesions, probably by the tuberculin-like products present. This turnover was accelerated by delayed hypersensitivity, which simultaneously increased the rate at which the new MN immigrants developed local cellular immunity. After two or three weeks the rate of developing this immunity in recently entering MN became so rapid that the bacillus was killed in their cytoplasm before they themselves were killed. At this stage the lesions began to regress and heal: the stimulating (and toxic) bacillary antigens were eliminated, and the macrophages present had a slower turnover.

This work has changed our conception of the pathogenesis of tuberculosis: formerly tuberculous lesions were thought to be rather static, and macrophages were thought to develop immunity gradually. Now such lesions are thought to be dynamic: macrophages constantly enter and constantly die, and the speed with which they develop immunity is of crucial importance. —(Adapted from authors' summary)

Kats, L. N., Gulevskaya, S. A., Zubok, L. P. and Nemsadze, M. N. Submicroscopic organization of *Mycobacterium tuberculosis*. J. Microbiol. Epidemiol. Immunobiol. N-4 (1972) 33-37. (In Russian, English summary)

An electron microscope study was carried out of the ultrathin sections of *Mycobacteria tuberculosis* strains H₃₇Ra (avirulent), H₃₇Rv (virulent) and BCG (attenuated). A microcapsule with an average electron density, more or less fringed in structure, was revealed in all the three strains under study; the cell wall consisting of electron-translucent layer and a layer of average electron density of homogeneous structure was found. Cytoplasmic membrane was closely attached to the cell wall often forming a single structure with the wall. Cytoplasmic membrane was found to have a three-layer structure in lysed or partially lysed cells. It formed membrane ring structures or structures of mesosome type, lamellar or tubulovesicular. The cytoplasm possessed a granular component, electron dense inclusion vacuoles, and a nucleoid. The cells divided by forming a transverse septum, a constriction of the type seen in gram-negative bacteria.

The ultrastructure of superficial layers of the cell is discussed in connection with the virulence and the presence of a cord-factor in *M. tuberculosis*. —(Adapted from authors' summary)

Kheifets, L. B. A method for determination of the ingestive capacity of macrophages obtained from experimental animals and man in relation to *Mycobacteria tuberculosis*. J. Microbiol. Epidemiol. Immunobiol. N-4 (1972) 37-43. (In Russian, English summary)

A method for determining the ingestive capacity of human macrophages obtained from the exudate of skin cantharidin blister on a model of *M. tuberculosis* is presented. The method, as well as determination of

ingestive capacity of peritoneal macrophages in a guinea pig, are based on the count of the ingested bacteria in conditions of a short-term experiment (40 and 90) without macrophage cultures. This method is of value for practical use. In comparison with the methods used for study of phagocytic activity of peripheral blood polymorphonuclear cells, the new method requires that at the end of exposure EDTA solution should be added in order to stop further phagocytosis, and the suspension washed off to get rid of extracellularly located bacteria. Comparative differences in phagocytic activity of macrophages of the two groups of animals may be masked by a potential use of the method of ranged differences in evaluation of the statistical significance of differences between the two groups compared.—(Adapted from author's summary)

Kondo, E. and Kanai, K. Further demonstration of bacterial lipids in *Mycobacterium bovis* harvested from infected mouse lungs. Jap. J. Med. Sci. Biol. 25 (1972) 105-122.

Mycobacterium bovis grown *in vivo* were separated by differential centrifugation from homogenates of the experimentally infected mouse lungs and subjected to lipid fractionation by the Anderson-Lederer method in comparison with the same strain grown on Sauton synthetic liquid medium. The percent yield of chloroform-soluble wax was much smaller than that of wax A in the lung bacilli. The esters of mycocerosic acid were the main components of wax A of the *in vivo* bacilli, but some esters of mycolic acid were not detected, unlike the case of the Sauton bacilli. An arabinolipid, probably arabinose mycolate, and tuberculostearic acid-containing cardiolipin were separated from bound lipids and phospholipid fraction, respectively, as the lipids common in both kinds of bacilli.—(Adapted from authors' summary)

Neiburger, R. and Youmans, G. P. An adaptation of the migration-inhibition technic for use in mice. Abstracts: Eighth

Annual Mtg. Reticuloendothelial Soc. (1971) 29.

Extensive work has been done in mice to study immunity to tuberculosis and disease due to other facultative intracellular parasites. However, in studies of lymphocyte products in cellular immunity, primarily guinea pigs have been used. It was, therefore, desirable to adapt the migration-inhibition technic for use with mouse cells.

Oil-induced peritoneal exudates were used to study the migration of cells from CF-1 mice immunized with the attenuated H37Ra strain of *M. tuberculosis*. Comparisons were made of immune and normal peritoneal exudates, in the presence or absence of antigen. Inhibition was limited to cells from H37Ra-immunized mice in the presence of PPD or H37Ra cells.

After demonstrating the direct inhibition of cells from immunized animals, we attempted to produce migration inhibitory factor (MIF). With the outbred CF-1 strain, we found that all supernatant fluids from stimulated or unstimulated lymphocyte cultures would inhibit the migration of normal mouse macrophages. Therefore, we used the inbred C57B1/6 strain in an attempt to eliminate the background activity. With this strain, we were able to demonstrate MIF production by splenic lymphocytes from immune animals stimulated in culture by PPD, but not by unstimulated immunized or stimulated or unstimulated normal lymphocytes.

Since MIF can be produced by lymphocytes from mice showing delayed hypersensitivity, it now becomes possible to study *in vitro* the relationship between delayed hypersensitivity and immunity to infection in mice.—(Adapted from authors' summary)

Tsukamura, M. The pattern of resistance development to rifampicin in *Mycobacterium tuberculosis*. Tubercle 53 (1972) 111-117.

Pattern of resistance development to rifampicin of tubercle bacilli is an obligatory 'single-step' pattern. There is only one phenotype of resistance to rifampicin. Tubercle bacilli are either susceptible or re-

sistant to this antibiotic, and there is no intermediate level of resistance between sensitivity and high resistance.

Spontaneous mutants resistant to rifampicin are found in strains previously not exposed to this antibiotic at a rate of 10^{-7} to 10^{-8} . The presence of a single-step pattern makes tests for rifampicin sensitivity simple.

An abrupt change from sensitivity to high degrees of resistance is found in cultures isolated from patients treated with rifampicin.—Author's Summary

Windhorst, D. B. and Katz, E. S. Normal enzyme activities in chronic granulomatous disease leukocytes. Abstracts: Eighth Annual Mtg. Reticuloendothelial Soc. (1971) 8-9.

Three families having children with chronic granulomatous disease of childhood (CGD) were investigated for hereditary patterns utilizing leukocyte function and metabolism assays. NADH oxidase and glutathione (GSH) peroxidase activities were then evaluated in the leukocytes from patients and selected family members. Basic

studies included bactericidal assay, tetrazolium dye (NBT) reduction, and C-1 glucose and formate oxidation with intact leukocytes.

The two male patients demonstrated X-linked inheritance in that their mothers and several female relatives were found to be carriers. One of the male patients had four maternal uncles who died in infancy with infectious processes suggestive of CGD. Two of these were twin boys with disseminated BCGosis.

The female patient's family showed no abnormalities.

Cyanide-insensitive NADH oxidized/minute/milligrams protein (five determinations on three patients). Control values were $1.70 \pm .20$ (eight determinations on four controls). GSH peroxidase activity of the female patient's cell was 65.5 μ moles NADPH oxidized/minute/milligrams protein, whereas the control value was 72.8. Thus, these patients' leukocytes do not conform with previously reported findings that NADH oxidase activity was deficient in CGD leukocytes and that GSH peroxidase was lower in two female CGS patients' leukocytes.—Authors' Summary