

CURRENT LITERATURE

This department carries selected abstracts of articles published in current medical journals, dealing with leprosy and other mycobacterial diseases. Abstracts are supplied by members of the Editorial Board and Contributing Editors, or are reproduced, with permission, from other abstracting journals.

General and Historical

✓ **Alfonso-Armenteros, J.** Sobre la supresión de la palabra lepra. [About the withdrawal of the term "leprosy."] *Rev. Cub. Med.* **10** (1971) 239-244.

The English summary appended to the paper is as follows. The author makes references to several propositions that have been presented, without success, in different international leprology congresses in order to eliminate the word *leprosy* from medical terminology for humanitarian reasons. He objects to such elimination from the viewpoint that no great benefit will be thus obtained from the belief that such change will lead to confusion. Likewise, the author argues that deficiencies in health education in *leprosy* are fundamentally caused by not putting into practice all knowledge about this disease.—(From Trop. Dis. Bull.)

✓ **Bergel, M.** Considerations on the pathogeny, prevention and treatment of leprosy. *Publ. Cent. Estud. Leprol.* **11** (1971) 91-115.

The author realizes a study of the pathogeny, prevention and treatment of leprosy. He points out the difference between leprosy, tuberculosis and rat leprosy.

He criticizes the employment of antibiotics and chemico-therapeutics in leprosy. He

makes a comparative study between the sulphones and sulphonamides. He describes the auto-oxidative disease. He makes a critical analysis of experimental leprology and of the transmission of leprosy in animals. He studies the inoculation of *M. leprae* in adipose tissue. He indicates future investigations to be realized. He gives guidelines on the prevention and treatment of leprosy.—(Adapted from author's English summary)

Dermatologia (Mexico) **15** (1971) 1-126.

This issue contains a discussion of diagnostic methods significant to the identification of cases of leprosy, by Obdulia Rodríguez, and a résumé of the May 13 meeting of the Asociación Mexicana de Acción Contra la Lepra, at which Gómez Vidal and Donaciano Fernández reported on 100 new cases studied at the Pascua center. Latapi reviewed George Hill's "Leprosy in Five Young Men," laconically wondering (at a later meeting) how many columns would be needed for 100 cases.—G. L. Fite

Jopling, W. H. Leprosy. *Practitioner* **207** (1971) 164-172.

A review, presenting standard information.—G. L. Fite

Clinical Sciences

✓ **Addy, J.** Systemic sclerosis masquerading as leprosy in Ghana. *Ghana Med. J.* **10** (1971) 218-222.

This account is of some interest in presenting the importance of correct diagnosis in some countries where a disease

masquerading as leprosy might lead to the introduction of the wrong person into a leprosarium with all the attendant stigmata. This article reports two cases of scleroderma, in which leprosy had been the erroneous diagnosis for some time. With leprosy the more common of the two diseases

in Ghana, the error emphasizes that not all that is hypopigmented or "trophic and clawed" is leprosy.—G. L. Fite

Bechelli, L. M. and Martinez Dominguez, V. Disability index for leprosy patients. *Bull. WHO* 44 (1971) 709-713.

This paper, written for statisticians, proposes the use of a disability index (DI) to compare the severity of disabilities in patients with leprosy in different areas of the same country, or in different countries or continents. Three types of disability indices are proposed: DI(1) to record maximum grades; DI(2) for all recorded disabilities; and DI(3) as a variant of the first.—W. H. Jopling (*Adopted from Trop. Dis. Bull.*)

Drutz, D. J. and Gutman, R. A. Renal tubular acidosis in leprosy. *Ann. Int. Med.* 75 (1971) 475-476.

Forty-nine leprosy patients and 30 normal control subjects were evaluated for renal acidification capability. In ten, the urine pH could not be lowered to less than 5.5 in response to the NH_4Cl stimulus. In contrast, the other 39 achieved a mean minimal pH of 5.24 ± 0.02 SD and the 30 controls achieved a mean of 5.1 ± 0.19 SD. The acidification defect was unrelated to the type of leprosy or other factors. Whatever the explanation, this has not shown itself to be a clinical problem.—G. L. Fite

Hobbs, H. E. and Choyce, D. P. The blinding lesions of leprosy. *Leprosy Rev.* 42 (1971) 131-137.

This paper is a résumé of the current views of the complications of leprosy which may lead to blindness. Two types of ocular lesions are responsible for the majority of cases of blindness due to leprosy.

Lagophthalmos from involvement of the facial nerve in the tuberculoid form of leprosy leads to exposure keratitis with vascularization, opacifications and loss of vision. This involvement of the cornea may be aggravated by trigeminal nerve damage in which corneal sensation is diminished or lost.

Intrinsic corneal lesions, lepromata or interstitial keratitis, abnormalities which are

apparent to the naked eye, are included in this group. Iris pearls and lepromata and the acute iritis in the lepra reaction may also be included, for these observations do not require specialized apparatus.

It is not disputed that the commonest cause of blindness in leprosy is an insidious form of iritis or iridocyclitis, the signs of which are often invisible to the naked eye. The eye may remain white without telltale ciliary flush, and the turbidity of the aqueous humour due to the exudation of leucocytes may, in the early stages, cause little hindrance of vision. The condition then progresses slowly but inexorably until atrophy of the iris occurs and exudate occludes the pupil and destroys sight.

The slit-lamp and corneal microscope enable the earliest signs of this type of disease to be detected and timely treatment may prevent disaster. This type of iritis is found predominantly in the lepromatous form of leprosy. The slit-lamp should be employed whenever possible in the routine examination of all patients with active disease.

Estimates of the amount of ocular involvement in leprosy and blindness vary from country to country. An account is given of an investigation of 507 patients at Sungei Buloh in Malaysia in a large leprosarium. The actual figures are worth quoting:

Ocular lesions—all types

32.5%

Leprotic eye lesions

50% of above

Leprotic iritis

50% of preceding figure

Total no of blind patients

36 (7.1% of sample)

Blind from leprotic lesions

18 (50% of total)

Blind from leprotic iritis

11 (61% of leprotic lesions)

The article is illustrated by eight excellent photographs.

[There is perhaps nothing new in this article, but it does emphasize a way in which personal and economic loss due to the blinding potentialities of leprosy could be mitigated. The saving of the sight of one patient is worth more than the cost of a

slit-lamp.

In the early 1930's, the writer of this abstract demonstrated by slit-lamp technic minute iris pearls to Professor Warrington Yorke and Dr. (later professor) Murgatroyd, both, alas, deceased. They did not, it is true, cry "Eureka!" but they were audibly impressed at this new approach. The patient was a European who had lived many years in Sarabajo and who manifested generalized signs of the lepromatous type of this disease. Relations with whom he came to live in this country attributed, he said, his leonine facies to many years exposure to the tropical sun.]—A. McKie Reid (*From Trop. Dis. Bul.*)

✓ **Inage, M.** Classification of creeping in leprosy patients and conditions of the use of the hands (Report I). *La Lepro* 39 (1970) 73-79.

Through the observation of moving actions in patients with severe functional injuries, it was found that the creeping action is generally performed in the TATAMI (floormat) living mode of Japanese life.

The author has attempted to investigate the causes and effects of "creeping action," classify the types of this action, and investigate the auxiliary action of the hands accompanying the creeping action. The creeping action itself is performed by three actions; crawling on all fours, buttock-chafing and kneeling. The auxiliary actions of the hands are classified as; palm-type, grip-type and dorsal hand-type, according to the way the hands are used.—(*Adapted from author's summary*)

✓ **Inage, M.** Relation between creeping in leprosy patients and lesions on the extremities together with loss of fingers (Report II). *La Lepro* 39 (1970) 80-87.

Many of the patients with leprosy who have been suffering from severe functional disorders in the lower extremities are mostly chronic cases afflicted previously to the present development of various therapies for leprosy. In many cases they suffer functional disorders of the upper extremities. Therefore, most of them have to move about the room by creeping from one place to another.

Their creeping movements are usually performed by three activities; crawling on all fours, buttock-chafing and kneeling. In detailed observation of these actions of creeping, it was found that the muscular effort is concentrated abnormally in the knee, buttock and upper extremities in the actions of "crawling on all fours" and "buttock-chafing" because of the necessity of the auxiliary action of the hands. Great muscular effort is concentrated in the knees while kneeling.

Based on such observations, studies were made on the relationship between the creeping action and the lesions in the four extremities together with loss of fingers—(*Adapted from author's summary*)

✓ **Inage, M.** X-ray findings in the bones and joints of creeping leprosy patients (Report III). *La Lepro* 39 (1970) 88-94.

The creeping action of leprosy patients is one of the important means for moving from one place to another for those patients with severe incapacity in the lower extremities. Those who perform the moving actions by crawling on all fours, buttock-chafing and kneeling are concentrating an abnormally great effort in the knee joints, ischium joints, hand joints, and elbow joints; therefore, x-ray pictures were taken for the observation of the bones and joints of these patients.

Some pictures showing new genesis of the bones were observed at some parts of the knee joints, also at the tibia nodes and those parts of the kneecaps which are muscle-tendon-attached, portions of the elbow joints, and in one case the elbow joints displayed symptoms resembling Charcot's disease.—(*Adapted from author's summary*)

✓ **Jordy, C. F. and Belda, W.** Consideracoes sobre a conducao nervosa periferica na hanseniose. [Peripheral nerve conduction in leprosy.] *Rev. Brasil Leprol.* 37 (1970) 61.

The authors measure the rate of conduction of the nerve impulses in 16 leprosy patients, all with clinical signs of peripheral neuropathy. Multiple areas were tested, yielding measurements of a total of 38

regions. Normal values were found throughout, and rarely unexpected high values were observed. In clinically undamaged areas no reductions in time were found, as have been observed in other types of neuropathy. (—From authors' summary)

✓ **Léon, A. P., Cano, C., Latapí, F. and Malacara, M.** Ulteriores investigaciones sobre el diagnóstico serológico de la lepra. II. Efecto del tiempo de evolución y tratamiento sobre el resultado de la reacción de fijación del complemento en comparación con la baciloscopia. [Serological diagnosis of leprosy. II. Effect of evolution of the disease and its treatment on the complement-fixation test in comparison with bacteriological findings.] *Rev. Invest. Salud Publica* 30 (1970) 217-236. (In Spanish, English summary)

The English summary appended to the paper is as follows. The complement fixation test with the polysaccharide isolated from *M. tuberculosis* by Léon *et al.*, as antigen was performed with the blood sera of 158 cases of leprosy of a second group studied. It was positive in 85.2% of 135 cases of lepromatous leprosy; in 61.5% of 13 cases of tuberculoid leprosy; in 4 of 8 (50%) indeterminate cases, and in all the 4 (100%) dimorphous cases studied. These results agree with those obtained in 107 confirmed cases of leprosy of a previous investigation. The CFT was positive in 100% of the cases of lepromatous leprosy with less than 5 years of evolution; the proportion of positive cases decreased slowly from the fifth year of illness down to 71.4 in cases with 30 or more years. In a greater proportion of cases the CFT became negative within the time duration of treatment with DDS; it decreased to 95.2% after one year and to 66.6% after 20 years of treatment. The statistical analysis by the χ^2 (chi square) test shows a significant association of negativization of the CFT with the time of treatment, particularly after 10 years of treatment. The bacilloscopy and the CFT became negative in geometrical proportion and directly with the time of treatment; but faster and more marked for the bacilloscopy. The difference between the rate of

positive bacilloscopies and positive CFT increased in geometrical and direct proportion with the time of evolution and the time of treatment, in favor of the CFT. The CFT was positive in almost 100% of the cases with negative bacilloscopy. The titers of the CFT decreased in geometrical proportion and inversely to the time of evolution and the time of treatment; but the rate of decrease was significantly greater with the time of treatment than with the time of evolution. It is concluded that the CFT with the antigen and the technic used in this work has a high diagnostic value for the diagnosis of leprosy and may be used to evaluate a drug or a therapeutic method for leprosy. (—From *Trop. Dis. Bull.*)

✓ **Maeda, M.** [On impedance of the skin of leprosy patients: a comparative study between the so-called skin of the leprosy patients and the normal skin of normal individuals.] *La Lepro* 30 (1970) 210-238. (In Japanese, English summary)

The English summary appended to the paper is as follows . . . (1) The impedance value is assumed to be closely related to the sweat function, and also has connection with insensible perspiration as well as blood circulation of the skin.

(2) The impedance value is regarded to be a very good index in expressing the skin function of the leprosy patients. Thus, the skin function of the so-called normal skin of the leprosy patients is conclusively supposed to be as follows: the skin of the trunk as well as the nape of the neck has a markedly increased skin function, while the skin function of the forearm and hand as well as the same of the leg and foot is greatly decreased in the leprosy patients than in the normal individuals. (—From *Trop. Dis. Bull.*)

✓ **Miranda, R. P. G.** Stomatological aspects in children suffering from Hansen's disease. *Publ. Cent. Estud. Leprol.* 11 (1971) 71-76.

This work has in view an investigation of the stomatological aspects of the mouth cavity of children suffering from leprosy in comparison with children not suffering from leprosy. —Author's Summary

Chemotherapy

✓ **Belda, W.** Resultados preliminares no tratamento da reacao hansenica com o dextrosulfenidol. [Preliminary results of treatment of leprosy reactions with dextrosulfenidol.] *Rev. Brasil Leprol.* **37** (1970) 5-16.

Ten cases of leprosy reactions were treated daily with 1.0 gram of dextrosulfenidol (thiamphenicol) in two divided doses of 0.5 gm each for seven days. Nine of the ten showed improvement, and the authors stress the immunosuppressive activity of the drug and need for further investigation.—G. L. Fite

✓ **Cline, M. J.** Drug potentiation of macrophage function. *Infection Immunity* **2** (1970) 601-605.

By subjecting cultured leucocytes containing ingested living *Listeria* to various concentrations of clofazimine [B 663, Lamprene (Geigy)] and of oxygen, the author shows that in aerobic conditions clofazimine potentiates bacterial killing by the macrophages. The potentiation observed corresponds to concentrations of the drug that are obtained in man when therapeutic doses are given. Even serum concentrations that are not high enough to be directly injurious to the organism used are somehow rendered bactericidal when the macrophages take up the drug. Clofazimine increased the oxygen consumption of the leucocytes; this increased utilization was not invalidated by potassium cyanide, an observation that suggests that the mechanism is independent of the mitochondrial system.—S. G. Browne (*From Trop. Dis. Bull.*)

✓ **Contreras Duenas, F.** Die Fruhdiagnose, Therapie und Forschung in der Lepra-bekämpfung. [Early diagnosis, treatment and research in the control of leprosy.] *Oeff. Gesundheitwesen* **32** (1970) 667-671. (In German, English summary)

With the growing incidence of leprosy in the world, in tropical lands as well as in the USA and some European countries, the

point is made that the three factors named in the title are essential prerequisites for eradication of leprosy. Identification of individual cases (and contacted persons) by the authorities as well as examinations of the surroundings are important for early diagnosis. *Type and duration of therapy* is discussed with reference to the possible complications: besides sulphas, particularly thalidomide (Contergan) for control of the leprosy reaction and recently Lampren B 663 for sulpha intolerance. The author refers to his first, satisfactory results with Lampren (Geigy) and oxygen overpressure treatment. He refers to some small problems for scientific research.—(*From Trop. Dis. Bull.*)

Cucinell, S. A., Israeli, Z. H. and Dayton, P. G. Microsomal N-oxidation of dapsone as a cause of methemoglobin formation in human red cells. *Amer. Soc. Trop. Med. Hyg.* **21** (1972) 322-331.

Methemoglobinemia is an important side effect of dapsone (4,4'-diaminodiphenyl sulfone; DDS) therapy. Previous workers had shown a correlation between methemoglobin formation and time of incubation of DDS in a system involving rat liver microsomes and human red cells (RBC). N-oxidation of ring ¹⁴C labeled DDS by rat liver microsomes was studied both in the presence and absence of RBC. Evidence is presented that *in vitro* DDS is metabolized to the monohydroxylamine of DDS (DDS-NOH), and maybe to the nitroso-(DDS-NO) and azoxy (azoxy-DDS) analogues of DDS. The latter compounds could also arise from non-microsomal oxidation of DDS-NOH. Specific isotope dilution procedures were employed to measure the N-oxidation of DDS. These involved conversion to azoxy-DDS or the formation of the pentocyanamine ferroate complex of DDS-NOH (and/or DDS-NO). The extent of total N-oxidation of DDS was always less when experiments were carried out in the presence of RBC than in their absence. This suggests that DDS-NOH is enzymatically reduced by RBC. Like other micro-

somal oxidations, N-oxidation of DDS was inhibited by SKF-525A. Our studies indicate that DDS-NOH (and/or DDS-NO) is the cause of the methemoglobinemia observed in dapsone therapy.—Authors' Summary

Ellard, G. A., Gammon, P. T., Rees, R. J. W. and Waters, M. F. R.—Studies on the determination of the minimal inhibitory concentration of 4,4'-diamino-diphenyl-sulfone (dapsone, DDS) against *Mycobacterium leprae*. *Leprosy Rev.* **42** (1971) 101-117.

Plasma was obtained from mice given 0.01% dapsone in their diet, and from mice injected with dapsone 10 and 50 mg/kg. The concentrations of dapsone, N-acetyl-DDS (MADDS) and N,N' diacetyl DDS (acedapsone, DADDS) were determined fluorometrically. In mice fed with dapsone the mean serum concentration was 0.74 µg/ml; in mice injected with dapsone 10 mg/kg, one hour after injection the mean concentration was 5.61 µg/ml and four and in the mice injected with dapsone 50 mg/kg, one hour after injection it was 256 µg/ml; mg/kg, one hour after injection the mean concentration was 23.9 µg/ml, and four hours after injection it was 14.3 µg/ml. The concentration of dapsone in the urine of patients with leprosy who received 1 mg dapsone daily was 0.075 µg/ml. Analysis revealed that dapsone was being consumed from no other source. Three hours after 1 mg dapsone the serum concentration "averaged 0.018 µg per ml." However, the authors admit that "it was not possible to measure individual DDS serum concentrations."

Since it is known that the multiplication of *Mycobacterium leprae* in the mouse is inhibited by feeding 0.0001% dapsone but not by 0.00001% dapsone, it is concluded that the minimum inhibitory concentration against *M. leprae* must be less than 0.01 µg/ml. Daily, 1 mg dapsone has been found to be satisfactory treatment for patients with leprosy, and in these patients the proportion of the drug found in the urine was similar to that found in patients treated with 50 mg or 300 mg dapsone daily. The half-life of dapsone in the mouse was 2.7 hours after dapsone 10 mg/kg, and

3.7 hours after 50 mg/kg. Thus, the exact time when serum concentrations of dapsone are estimated after very low daily dosage is critical in establishing whether the blood level of dapsone is adequate. The significance of these findings is discussed in detail.—C. S. Goodwin (*Adapted from Trop. Dis. Bull.*)

Imkamp, F. M. J. H. Lampren (clofazimine), *Med. J. Zambia* **4** (1970) 69-73.

This is a report of favorable experience with clofazimine (Lamprene, B 663) in the management of recurrent lepra reaction (*erythema nodosum leprosum*), and six case histories are given. The drug also has a good anti-leprosy effect, even at the low dosage of one capsule (100 mg) three times a week, but for the control of lepra reaction the dosage varies between 100 and 300 mg daily according to the patient's need. The only side-effects encountered were diarrhea and darkening of the skin; diarrhea responds to temporary suspension of treatment and the pigmentation gradually fades when the drug has been stopped.—W. H. Jopling (*From Trop. Dis. Bull.*)

Iyer, C. G. S., Languillon, J., Ramanujam, K., Tarabini-Castellani, G., de la Aguas, J. T., Bechelli, L. M., Uemura, K., Dominguez, V. M. and Sundaresan, T. WHO coordinated short-term double-blind trial with thalidomide in the treatment of acute lepra reactions in male lepromatous patients. *Bull. WHO* **45** (1971) 719-732.

The treatment of lepra reactions constitutes one of the most serious problems in leprosy. For this reason, the first reports in 1965 of the favorable results obtained with thalidomide aroused considerable interest and led WHO, in 1967, to carry out a trial with the cooperation of four centers. A short-term double-blind trial was designed to study the effect of thalidomide, in comparison with acetylsalicylic acid, in the treatment of acute lepra reactions in male lepromatous patients. Acetylsalicylic acid was used instead of a placebo because of its antipyretic and analgesic activity. Because of the severe adverse reactions that may be caused by thalidomide, mainly the terato-

genic effects, only males were included in the trial.

The results of this short-term study seem to confirm previous reports of the efficacy of thalidomide and indicate that acetylsalicylic acid also seems to be helpful in the management of certain symptoms of lepra reactions.—(Adapted from authors' summary)

Karat, A. B. A., Jeevaratnam, A., Karat, S. and Rao, P. S. S. Controlled clinical trial of clofazimine in untreated lepromatous leprosy. *Brit. Med. J.* 4 (1971) 514-516.

A comparison of clofazimine and dapsone in the management of untreated lepromatous leprosy showed no significant differences between the two drugs in terms of morphologic and bacterial indices. The incidence of *erythema nodosum leprosum* was similar in the two groups. Since dapsone is cheaper than clofazimine it remains the drug of choice for the routine management of untreated lepromatous leprosy.—Authors' Summary

Languillon, J. Note préliminaire sur le traitement de la lèpre par la rifampicine. [A preliminary note concerning treatment of leprosy with rifampicin.] *Med. Afr. Noire.* 18 (1971) 273-281.

Very satisfactory clinical and bacteriological improvement is reported by the author, who treated 22 patients suffering from lepromatous leprosy with rifampicin at a dose of 900 mg daily for one year. The Morphological Index fell from 80% to zero in five months, and the localized and diffuse lepromatous infiltration diminished, as shown convincingly by the accompanying black and white photographs in the article.—S. G. Browne (From *Trop. Dis. Bull.*).

Leiker, D. L., Blenska, W., Carling, D., Fitzherbert, M. and Larssen, P. Bacteriological effect of Lamprene (clofazimine) in lepromatous leprosy. (Report of one year's treatment of 44 patients with 100 mg of Lamprene daily.) *Leprosy Rev.* 42 (1971) 125-130.

At four leprosy hospitals in Africa

(Uganda, Nigeria, Ethiopia and Tanzania) 44 patients with lepromatous leprosy were treated with 100 mg of clofazimine daily. [It is not stated how many patients were treated in each area.] Histologic examination was performed on skin biopsy specimens sent to the Royal Tropical Institute in Amsterdam. All patients were classified as lepromatous; patients with borderline features being excluded. Each patient had a density of bacilli of "4+" (Ridley-Cochrane scale) and at least 20% solid staining bacilli. In the table a differentiation is shown between "intact" and "fragmented" bacilli. Many patients had no intact bacilli and in others the percentage ranged from 1 to 9. The percentage of fragmented bacilli ranged from 21 to 90. [It is not clear how the figure of 20% solid staining bacilli is reached.] After three months, three patients alone had intact bacilli. The patients were treated for 12 months and this treatment "with few exceptions" was continued during periods of reaction. Eighteen patients had "repeated severe reactions in the year prior to the beginning of treatment with clofazimine." Serial skin biopsy specimens were taken at three month intervals from the same lesion, and all specimens were examined by the same investigator. The percentage of "granular" bacilli increased from 50 to 80 after three months and to 95 after six months. The authors conclude that the bacteriological effect of this treatment is "comparable with that of the sulphones."—C. S. Goodwin (Adapted from *Trop. Dis. Bull.*)

Marques, M. B., and Opromulla, D. V. A. A talidoma no tratamento da reacao leprotica. [Thalidomide in the treatment of the lepra reaction.] *Rev. Brasil Leprol.* 37 (1970) 42-49.

The authors report the treatment of 425 patients with reactive phases of leprosy during a five year period, with favorable results finding that it has great effectiveness in controlling reactions. The standard dose was 100 mg daily, with some increase in patients not responding by the fifth day. The sedimentation test was used to control the medication, and oral contraceptives are recommended if it is necessary to adminis-

ter it to a potentially pregnant woman.—G. L. Fite

✓ **Matsuo, Y.** Activity of thiozamin [*p*-(*p*-aminobenzenesulfonyl) - benzaldehyde thiosemicarbazone] against *Mycobacterium leprae* infections in mice. *Jap. J. Microbiol.* **15** (1971) 93-94.

Multiplication of *Mycobacterium leprae* in mouse foot pads was completely suppressed by thiozamin 1 mg/day thrice weekly administered orally, when the treatment was started on the day of infection and continued for six or twelve months. When treatment was begun six months after infection and continued for three months, the harvest twelve months after infection was reduced to 4.5×10^5 organisms compared with 1.3×10^6 organisms in untreated mice. The number of mice in each group is not mentioned.—C. S. Goodwin (*From Trop. Dis. Bull.*)

✓ **Miranda, R. N. and Cunha de Paula, B. S.** Effect of oxytetracyclin on mycobacteria cultures isolated from leprosy patients. *Publ. Cent. Estud. Leprol.* **11** (1971) 77-84.

1. Oxytetracyclin, in concentrations ap-

proximate to those recommended for human blood, provokes an evident macroscopic alteration of coloring in cultures of mycobacteria obtained from leprosy patients.

2. Also on the microscopic level, it causes a greater agglutination of bacilli, as though it were a self-defensive mechanism of the same.

3. The mycobacteria treated with oxytetracyclin immediately after replanting do not grow, showing the acting inhibition of the antibiotic *in vitro*.

4. Those of the previous item that manage to develop, do so at the longitudinal extremes of the medium, this escape evincing that the zone attacked by the antibiotic is really unfavorable to reproduction.

5. Replants of the cultures treated with the antibiotic do not show any development, leading to the belief that there had occurred an alteration in their biochemistry.

6. Both the *M. tuberculosis* and the Stefansky bacillus in our experiment showed that they possessed some resistance to the action of oxytetracyclin.

7. The age of the cultures and the concentrations of oxytetracyclin did not influence the results of the experiments.—(*Adapted from authors' conclusions*)

Immuno-Pathology

✓ **Abe, M., Yoshino, Y., Kobayashi, S. and Hokibara, H.** Studies on the preparation, standardization and preservation of lepromin. VII. Changes in the bacterial count and the potency of lepromin during preservation. *La Lepro* **39** (1970) 1-6. (In Japanese, English summary)

For the standardization of lepromin, it is necessary to know its period of potency, and also to run a trial on prolonged preservation by the freeze-dry procedure. A batch of lepromin was divided in two parts; one part was lyophilized without addition of phenol and preserved in a refrigerator for three and five years, while the other part was kept, as a suspension containing phenol, in a refrigerator for the

same period. After storage, the lyophilized lepromins returned immediately to stable suspensions with the addition of 0.5% phenol solution. The bacterial counts of these lepromins, using the pinhead method described by Hanks *et al*, were not different from the standard value, i.e., 150-160 million per ml, while that of the lepromins kept in a refrigerator were reduced to about 74% and 70% of the original count after three and five years, respectively.

Comparative tests with these lepromins were carried out by examining the size of the Fernandez and Mitsuda reactions in leprosy patients, the results being shown in Tables 2 and 3. It was found that the Mitsuda reactions caused by lepromin preserved as a suspension for five years

were clearly weaker than that of the lepromin lyophilized for the same period, and that the Fernandez reactions were not different for the two lepromins. In the case of the lepromins preserved for three years, there was no significant difference in potency with respect to the Fernandez as well as the Mitsuda reactions.—(*Adapted from authors' summary*).

Almeida, J. O. *et al.* Immunological problems in leprosy research. *Bull. WHO* 43 (1970) 879-890.

This important memorandum should be read in full by all wishing to keep abreast of current thinking in a field of leprosy research in which considerable and significant advances are being made and will doubtless be made in the future. The wide range of pathological phenomena observed in clinical leprosy—extending from the self-limiting and self-healing forms of indeterminate leprosy to the rapidly advancing mycobacteriosis of lepromatous leprosy—may be regarded as an expression of the absence or presence of specific cell-mediated immunity to *Mycobacterium leprae*. Patients suffering from lepromatous leprosy frequently show lack of such immunity to other antigens. On the other hand, circulating antibody response is high or very high in these patients, which suggests that humoral antibodies play little or no part in resistance to infection. The role of allergy in the development of the lesions of *erythema nodosum leprosum* is considered in the light of the recent demonstration of deposited immunoglobulin and complement in skin examined by the fluorescent antibody technic.

The experimental animal is providing a useful model for the study of immunological responses, both normal and thymectomized-irradiated animals contributing in this way to the pool of knowledge. The importance of the lymphocyte and the macrophage in experimental and clinical infections is recognized.

The memorandum gathers usefully within a concise compass various techniques concerned with determining the immune response in patients suffering from various clinical types of leprosy, and makes sugges-

tions to improve the precision and comparability of results—S. G. Browne (*From Trop. Dis. Bull.*)

Blumberg, B. S., Melartin, L., Guinto, R. and Lechat, M. Lepromatous leprosy and Australia antigen with comments on the genetics of leprosy. *J. Chron. Dis.* 23 (1970) 507-516.

The intriguing observation that Australia antigen (Au (1)) is associated with the occurrence of lepromatous leprosy (*Trop. Dis. Bull.* 64 [1967] 1330) is further investigated in the studies reported in this paper from Cebu (Philippines).

The serum findings in a larger group of patients confirm the initial conclusions that Au (1) is found in a significantly higher proportion of patients with lepromatous leprosy than in those with tuberculoid leprosy or in the general population; that it is found in a higher proportion of males than females, and that the highest frequency occurs in the age-group 6-9 years.

The significance of these observations is discussed, and the hypothesis advanced that the postulated gene (which "in double dose" confers increased susceptibility to hepatic virus infections) may also confer susceptibility to other chronic infections, including leprosy. If this is the case, lepromatous leprosy would follow a pattern of autosomal recessive inheritance in areas of high leprosy prevalence. Persons carrying this gene would, if exposed to leprosy infection, be more likely to develop the lepromatous type than those not carrying the gene. Susceptibility is not on these grounds absolute, but such factors as age and sex and possibly other genes are also involved.—S. G. Browne (*From Trop. Dis. Bull.*)

Bovornkitti, S., Ramasutra, T. and Chantarakul, N. Skin reactivity to mycobacterial antigens in leprosy patients. *J. Med. Ass. Thailand* 53 (1970) 338-345.

Three hundred and thirty-three patients with lepromatous leprosy, 144 with tuberculoid and eight with borderline leprosy were tested with old tuberculin and three mycobacterial antigens obtained from "photochromogenic, scotochromogenic and

nonchromogenic acid-fast bacilli.' A dilution of 0.1 ml of 1 in 1,000 of each of the antigens was "simultaneously applied intracutaneously." Palpable induration of 5 mm or greater was regarded as positive. Four tables and a histogram give the results. Of the patients, 80% gave a positive reaction with old tuberculin, 69% with the photochromogen, 9% with the nonchromogen and 4% with the scotochromogen antigen. Of 416 "normal" Thai subjects, 94% gave a positive reaction with old tuberculin, 76.5% with the photochromogen, 5% with the nonchromogen and 1.5% with the scotochromogen antigen. Twenty-five patients were negative to old tuberculin but positive to the photochromogen antigen, and 43% of photochromogen reactors exhibited responses stronger than those to tuberculin. These latter two findings are different from those in normal subjects. The percentage of patients with lepromatous leprosy who have positive reactions was slightly lower than the percentage in patients with tuberculoid leprosy. Histological pictures of skin reactions were the same as those seen in healthy persons.

The authors suggest that there is a "stronger cross-sensitization between nonchromogen and scotochromogen" (and photochromogens) to the *Mycobacterium leprae* than to *Mycobacterium tuberculosis*.—C. S. Goodwin (From Trop. Dis. Bull.)

Bullock, W. E., Jr., Ho, M. F. and Chen, M. J. Studies of immune mechanisms in leprosy. II. Quantitative relationships of IgG, IgA, and IgM immunoglobulins. J. Lab. Clin. Med. 75 (1970) 863-870.

Sera from 158 Chinese patients with lepromatous leprosy, 35 patients with tuberculoid leprosy, 22 Chinese hospitalized for long periods for neurological or cardiovascular disease and 25 "normal" Chinese were studied.

The serum levels of IgG, IgA and IgM were assayed by agar diffusion. The levels of all three immunoglobulins, but particularly IgA, were significantly higher in patients with lepromatous leprosy than in normal subjects. In patients with tuberculoid leprosy the levels were normal. In

patients with lepromatous leprosy who had been treated for more than two years the levels of IgA and IgG were highest, and in this group those who had suffered reactional episodes had significantly higher levels than those who had not had such episodes. The mean IgA level in the former was 715 mg%, and the latter 551 mg%, while in normal subjects it was 323 mg%. The IgM levels were highest in patients with lepromatous leprosy who had been treated for less than 12 months. The authors suggest that the sustained high immunoglobulin levels in patients with lepromatous leprosy may be due to the presence of dead but still immunogenic *Mycobacterium leprae*.

(For Part I, see Trop. Dis. Bull. 65 [1968], abstr. 1650)—C. S. Goodwin (From Trop. Dis. Bull.)

Carayon, A. Physiopathologie de la névrite hansénienne et bases thérapeutiques (nouvelle approche). [Physiopathology of leprosy neuritis and bases for therapy (new approach).] Med. Trop. 31 (1971) 503-523. (In French)

This detailed and well-documented study of the physiopathology of peripheral neuritis in leprosy embodies the investigations that the author has carried out over the past few years. In particular, the determinative role of constrictive fibrous tunnels and osteoligamentous narrowings on the whole pathology of leprosy neuritis is supported by clinical observations, perineural lymphography and arteriography with micronized lipiodol, and operative exposure. The vascular and lymphatic occlusion, the slowing of the radio-opaque flow, the ten-fold retardation in the absorption of the injected lipiodol—all indicate the importance of the constriction. In addition, localized arterial spasm reduces the blood flow to the constricted nerve segment.

The commonly affected peripheral nerves are studied in turn, with a wealth of practical detail, and the importance in each case of the anatomical constrictions is demonstrated. Other germane factors, such as the elongation and hence compression of the ulnar nerve when the elbow is flexed, and the presence of a zone of noninflamma-

tory edema distal to the site of constriction, are investigated and recorded.

The author attempts to correlate the sites of maximum-observed damage in the peripheral nerves with the liberation of specific substances from dead *Mycobacterium leprae*, myelin and Schwann cells, together with enzymes released from leucocytes as the result of chemotherapy.

The bases for rational therapy of threatened or actual peripheral nerve damage are discussed in the concluding section. The author favors a bacteriostatic drug, coupled with surgical release of constricting bands if present.

[This paper covers a vast field in rather summary fashion, touching lightly and provocatively on such specialized realms as the biochemistry and immunology of neuropathology. Unfortunately, no references are given to the numerous works cited.]—S. G. Browne (*From Trop. Dis. Bull.*)

Del Ray Calero, J. and Calbo Torrecillas, F.

Estudio en la lepra de la hipersensibilidad de base celular mediante el "test" de la inhibición de la migración linfocitaria (M.I.T.). [The lymphocyte migration inhibition test in leprosy.] *Rev. Sanid. Hig. Publica* 44 (1970) 1319-1332. (In Spanish, English summary)

The English summary appended to the paper is as follows . . . 1. Retarded hypersensitivity has been studied in 33 patients with diagnosis of leprosy. MIT technic (inhibition of lymphocyte migration) has been used with cultures in Mackais' plates.

2. Different antigens have been used for the study of M.I. test: lepromin, P.P.D. and different carbohydrate and lipid fractions of old tuberculin as well as different extracts of connective tissue: GPS (structure glycoproteins), CTC (proteins of soluble skin free from soluble collagen fraction by dialysis) and TCA (fraction containing soluble antigen extracted with trichloroacetic acid).

3. The results refer to different percentages according to the type of leprosy. Appropriate dilutions of antigens have been established for the macrophage inhibition test.

4. According to a global study the GPS Ag. has given an inhibition in 56.5% of cases; the CTC Ag. in 53.1% and TCA Ag. in 56.6%; antigenic fractions of connective tissue of structure glycoproteins type and soluble collagen extracted with TCA are the most positive ones.

5. Lepromatous patients whose immunity of cellular basis is depressed have weaker positive results against lipid or carbohydrate fractions of tuberculin. These results were higher against protein fractions which means that other factors are detected by MIT.

In tuberculoid and lepromatous groups a positivity of 60% and 31.25% respectively has been obtained for lepromin.—(*From Trop. Dis. Bull.*)

Alfonso-Armenteros, J. El examen neurológico de la lepra. [The neurological examination of leprosy.] *Rev. Cub. Med.* 10 (1971) 521-540. (In Spanish, English summary)

The author makes reference to the importance of the neurological examination in leprosy and argues that any clinical history of this disease cannot be considered as complete if exploration of the peripheral nerves is not made. Likewise, he exposes the alterations caused by leprosy on such nerves, which can create sensitivity, motor and trophic troubles and also vasomotor and pylomotor modifications and alterations in the secretion of sweat. All these changes are described by the author, both from the clinical aspect and the verifying tests. He also refers to the biopsy of the superficial nerves as a useful method for establishing the diagnosis in doubtful cases, although occasionally—he says—the histologic image is not sufficiently characteristic to deduce definitive results. He points out the need to perform the biopsy following a rigorous technic in order to avoid the definitive paralysis which an improper surgical act could cause. He ends the exposition with the differential diagnosis of the neurological processes of leprosy and several affections which present certain alterations of the peripheral nerves.—(*From Trop. Dis. Bull.*)

Enna, C. D., Jacobson, R. R. and Rausch, R. O. Bone changes in leprosy: A correlation of clinical and radiographic features. *Radiology* **100** (1971) 295-306.

Characteristic bone lesions in leprosy affect the small bones of the face, hands and feet. They are due directly to leprosy infection and indirectly to injurious effects of trauma and infection imposed upon denervated tissues. The manifestations of leprosy bone infection vary from the presence of bacilli in the bone to actual destruction. Distal absorption involves fingers and toes with the loss of digits. Combined absorption of length and width of bone often affects the metatarsophalangeal joints. Tarsal absorption patterns are determined by abnormal weight-bearing forces and infection.—Authors' Summary

Ghosh, S. and Mukherjee, A. T. ABO group distribution amongst leprosy patients. *Bull. Calcutta Sch. Trop. Med.* **18** (1970) 47-48.

Considering the distribution of blood groups amongst normal individuals, those of group "O" have got a greater susceptibility to leprosy infection and group "B" less. No such difference, in comparison to the normal, was observed in people belonging to blood groups "A" and "AB."—(From *Trop. Dis. Bull.*)

Godal, T., Rees, R. J. W. and Lamvik, J. O. Lymphocyte-mediated modification of blood-derived macrophage function *in vitro*; inhibition of growth of intracellular mycobacteria with lymphokines. *Clin. Exp. Immun.* **8** (1971) 625-637.

Lymphocytes, obtained from a human being or animal who has developed a specific immunity, when exposed to the specific antigen release substances called lymphokines. The authors obtained leucocytes from the blood of rabbits and men, and removed the majority of the lymphocytes and granulocytes. Of the remaining cells, "80%-90%" were monocytes which, when cultured, developed into macrophages. Mixed leucocyte cultures (MLC) were prepared by mixing equal numbers of leu-

cocytes from two rabbits. When supernatants from these MLC were added to a culture of macrophages obtained from one of these rabbits, the yield of macrophages after eight days was increased from 3×10^3 to 9×10^3 cells, and the macrophages developed intercellular cytoplasmic bridges and giant cells. Leucocytes were cultured from pairs of patients with tuberculoid and lepromatous leprosy. To a culture of macrophages, obtained from one of the patients with tuberculoid leprosy, were added killed *Mycobacterium leprae*. When MLC supernatant was added, the yield of macrophages after ten days was increased from 4×10^3 to 18×10^3 cells, and the macrophages developed intercytoplasmic bridges and giant cells. However, if lymphocytes were initially removed from the MLC, the supernatant did not stimulate multiplication. Macrophages from the patients with lepromatous leprosy when exposed to *M. leprae* and then to MLC supernatant did not increase in numbers. *M. microti* and *M. lepraemurium* multiplied in rabbit macrophages in the presence of unmixed leucocyte supernatants, but were inhibited in the presence of MLC supernatants. With filtered and unfiltered supernatants the results were identical. The authors state that these observations demonstrate clearly the lymphocyte dependence of macrophage proliferation. Also, the inhibition of intracellular multiplication of mycobacteria observed in this study indicates that the lymphokine mediated lymphocyte-macrophage interaction could be a potent antibacterial principle, at least against slowly growing intracellular bacteria. They also suggest that "there is an impairment of the *M. leprae* induced lymphocyte-macrophage interaction in patients with lepromatous leprosy." [These revealing experiments open new fields for leprosy research, as well as providing fundamental information on lymphokines.]—C. S. Goodwin (From *Trop. Dis. Bu.*)

Godal, T., Myklestad, B., Samuel, D. R. and Myrvang, B. Characterization of the cellular immune defect in lepromatous leprosy: A specific lack of circulating *Mycobacterium leprae*-reactive lymphocytes. *Clin. Exp. Immun.* **9** (1971) 821-831.

The leucocytes from the two groups were studied simultaneously and cultivated in the same pool of normal human serum. While the leucocytes from twenty-eight tuberculoid patients responded quite strongly to *Mycobacterium leprae* after seven days of culture (average lymphocyte transformation 11.1%), there was a complete lack of response in similar cultures from twenty-seven lepromatous patients (average 0.1% transformed cells). These results were confirmed by studies on cellular incorporation of ³H-thymidine in the cultures from four tuberculoid and four lepromatous patients.

This lack of response was quite specific as leucocytes from several lepromatous patients responded to BCG. Furthermore, four patients with both lepromatous leprosy and tuberculosis responded as strongly to BCG and PPD as tuberculous patients without leprosy.

The blastogenic response of purified lymphocytes to *M. leprae* revealed a similar pattern, i.e. the tuberculoid cells responded well, while again there was a lack of response in the lepromatous group.

It is concluded that the lepromatous patients lack circulating lymphocytes responding to *M. leprae*, indicating that their immunological defect as observed in the present study has features in common with immunological tolerance.—Authors' Summary

Kapoor, K. K., Gupta, S. C., Mehrotra, T. N. and Issac, J. J. Electrophoretic pattern of serum proteins in leprosy. *Indian J. Med. Res.* 59 (1971) 1424.

Serum proteins were studied in 25 normal healthy individuals and 193 patients suffering from various clinical forms of leprosy. Out of 193 leprosy cases, 71 were suffering from tuberculoid, 17 from dimorphous, 73 from lepromatous type of leprosy and 32 patients were showing lepra-reaction. Total serum proteins and gamma globulin fraction were found to be significantly increased in all the types of leprosy-tuberculoid, dimorphous, lepromatous and in lepra-reaction. In dimorphous type alpha-1-globulin was also decreased. In lepromatous type and in patients with lepra-

reaction albumin was found to be markedly reduced. The alterations in the total serum protein and its different fractions in different clinical forms of leprosy do not appear to be of diagnostic value.—Authors' Summary

Merklen, F. P. and Potier, J. C. Provocation par bacilles extraits de lépromes murins de la contraction d'un segment d'iléon de cobaye sensibilisé par sérum de lèpre humaine. [Induction by bacilli extracted from murine lepromata of the contraction of a portion of ileum of guinea pigs sensitized with serum from human leprosy.] *Bull. Soc. Path. Exot.* 63 (1970) 429-431.

The senior author had done previous work on the sero-diagnosis of leprosy by an immunofluorescent technic with *Mycobacterium lepraemurium* as antigen (Merklen and Cottenot, *Trop. Dis. Bull.* 67 [1970] abstr. 2575). In the present communication, the authors give details of a method they developed for sensitizing a portion of ileum by immersing it in the serum to be assayed for the presence of specific antibodies. When a suspension of *M. lepraemurium* of the necessary concentration is added to the sensitized loop of ileum (previously shown not to contract when immersed in normal human serum), the loop contracts and the contractions are registered on a smoked drum.

The authors consider that this result runs parallel to the lymphoblastic transformations of lymphocytes which they have shown to occur in human leprosy.—S. G. Browne (*From Trop. Dis. Bull.*)

Miranda, R. N., Cunha de Paula, B. S. and Zanotto, I. Cytology of pus in leprosy. *Publ. Cent. Estud. Leprol.* 11 (1971) 63-70.

1. The pus formed on the level of acute lepromatous leprosy during the clinical happenings improperly called "leprotic reaction," or, worse still, *erythema nodosum leprosum*, having the appropriate characteristics of that which is formed by acute processes.

2. This pus consists principally of polymorphonuclear leucocytes with a predominance among those of *neutrophils* in accordance with its cytological composition as detailed in the text of the work.

3. The polymorphonuclear leucocytes contained in the pus of leprosy lesions show up sometimes intact, sometimes degenerate, and, as an important finding, also vacuolized.

4. When the vacuole is present it is generally alone and big, and may be bigger than the diameter of the cell.

5. In the investigation made, these vacuoles showed up sometimes empty, sometimes containing globi of *M. leprae*. These globi may have various conformations, i.e., from those showing completeness down to those represented by bacillus fragments. In the latter case they occupy a greater part of the vacuolar space.

6. It was of great interest to note, in confirmation of previous findings of one of the authors, that the polymorphonuclear leucocytes are able to encompass *M. leprae*, which, in the interior of the cell, after a probable growth, shows various phases of degradation until disappearance.

7. It was found that among the polymorphonuclear leucocytes, the neutrophils are those which encompass *M. leprae* in the skin processes of suppuration in leprosy.—(Adapted from authors' conclusions)

Navalkar, R. G. Immunologic studies on leprosy. 1. Demonstration of antibodies in sera of leprosy patients using mycobacterial antigens. Jap. J. Exp. Med. 40 (1970) 467-472.

The author, using an antigen derived from *Mycobacterium smegmatis*, identified the "beta-precipitin" and, using an antigen from *M. kansasii*, the "delta-precipitin," in sera of patients with leprosy and tuberculosis, but not in the sera of those with syphilis, nor in healthy persons, nor in contacts of patients with leprosy. "Anti-beta" antibodies were found in 23 of 26 patients with lepromatous, in 10 of 17 with dimorphous and 3 of 56 with tuberculoid leprosy. "Anti-delta" antibodies were found in 22 of those with lepromatous, in 12 of those with dimorphous and in 21 of those with tuberculoid leprosy.—C. S. Goodwin (From Trop. Dis. Bull.)

Microbiology

Draper, P. and Rees, R. J. W. Electron-transparent zone of mycobacteria may be a defense mechanism. [Correspondence] Nature (London) 228 (1970) 860-861.

The authors have seen fibrillar structures closely associated with the cell-wall of *Mycobacterium lepraemurium* growing in the liver and spleen of infected mice. These could be demonstrated under the electron microscope after negative staining with either sodium silicotungstate or ammonium molybdate or after shadowing with platinum.

They are similar to structures seen in preparations of *M. lepraemurium* and reported personally to the authors by L. Sula of the Tuberculosis Research Institute, Prague. They are said to resemble, morphologically, the wax Ds from the cell-walls and from culture supernatants of *M. bovis* and from the BCG strain of this organism

(White. In: Molecular and Cellular Basis of Antibody Formation. Ed., Sterzl, 1965, p. 71, Prague: Czechoslovakian Academy of Sciences; Imaeda *et al.* J. Med. Microbiol. 2 [1969] 181.)

"It seems likely" that this fibrillar material is of bacterial rather than of host-cell origin, and that it occupies the "electron-transparent zone" previously discussed by Allen *et al.* (Trop. Dis. Bull. 52 [1965] 540) and also by others. J. A. Armstrong has reported, to the present authors, an electron-transparent halo around tubercle bacilli growing in mammalian cells; Imaeda (Internat. J. Leprosy 33 (1965) 669; and Trop. Dis. Bull. 63 [1966] 765) has seen something similar around *M. leprae* in Virchow cells.

Since the wax-like substance, thought to occupy the electron-transparent zone, is not digested by host cells the present authors

suggest that this "inert and mechanically protective material may explain the resistance of all these mycobacterial pathogens to killing by phagocytic cells."—P. Cavanagh (*Adapted from Trop. Dis. Bull.*)

Fisher, C. A. and Barksdale, L. Elimination of the acid-fastness but not the gram positivity of leprosy bacilli after extraction with pyridine. *J. Bact.* **106** (1971) 707-708.

Bacilli from a number of tissues excised from cases of lepromatous and borderline leprosy, unlike mycobacteria, were found to lose their acid-fastness after treatment with pyridine.—(*From Trop. Dis. Bull.*)

Leiker, D. L. Assessment of bacteriological changes in leprosy, based on serial biopsies. *Leprosy Rev.* **42** (1971) 121-124.

After a discussion of some of the advantages and disadvantages of the scraped-incision smear technic to estimate changes in bacterial numbers and staining, the author describes the collection of skin biopsy specimens and histological assessment of bacilli. His preference is for serial specimens taken from one large leprosy skin lesion, using a 4 or 6 mm punch. Fixation is in 4% formalin and all specimens are posted to him. The TRIFF method is used for routine staining and the Fite-Faraco-Wade technic is used to assess the numbers and staining of bacilli. Three categories of bacilli are distinguished: first, those that are completely and evenly stained; second, those that are unevenly stained but not yet "granular"; and third, bacilli that appear as granules. The author suggests that his method is the most satisfactory for the assessment of the "bacteriological effect of treatment" in drug trials.—C. S. Goodwin (*Adapted from Trop. Dis. Bull.*)

Levy, Louis. The effect of freezing and storage at -60°C on the viability of *Mycobacterium leprae*. *Cryobiology* **8** (1971) 574-576.

Suspensions of *M. leprae* in 10% glycerol and 0.1% bovine serum albumin were quick-frozen and stored for various periods

of time at -60°C . Viability of the bacterial suspensions, measured by means of Shepard's mouse foot pad technic, did not decrease progressively during prolonged storage, suggesting that the observed decreases in viability resulted from the freezing process rather than from storage. Considerable variability was noted in the loss of viability which resulted from freezing.—Author's Summary

Lu-Huynh-Thanh, Borges, M. and Coudert, G. Étude en microscopie électronique de mycobactéries cultivées provenant de lépromes humains. I. Observations morphologiques. [Electron microscope study of mycobacteria grown from human lepromas. I. Morphologic study.] *Bull. Soc. Path. Exot.* **2** (1971) 152-175.

Two strains of bacilli (Vi 1 and Rho 2) isolated from human lepromas and cultured *in vitro* were inoculated into mouse foot pads and studied with the electron microscope in biopsy. The infrastructure of the two strains was essentially identical, showed the features of mycobacteria, and occupied the cytoplasm of infected cells. They appeared more to be related to *M. leprae* than *M. lepraemurium*.—(*From authors' summary*)

II. Remarques sur diverse structures particulières. [Comments on characteristic structures.] *Ibid.* 176-189.

Some lamellar formations in apparently normal organisms seem to represent physiological variants. Others, on the contrary, present pictures found in older bacteria, which may signify decay of the bacilli in which they occur. It is supposed that the abnormal lamellar structures arise from circumstances within the bacillary bodies, rather than from influences of the culture medium.—(*From authors' summary*)

McRae, D. H. and Shepard, C. C. Relationship between the staining quality of *Mycobacterium leprae* and infectivity for mice. *Infection Immunity* **3** (1971) 116-120.

The proposal has been made that only solidly staining forms of *Mycobacterium*

leprae are viable. On the basis of a previous study, solidly staining bacilli were defined as those that stained completely and darkly throughout their length. A study was carried out to correlate the proportion of solidly staining bacilli in inocula with the infectivity and rate of appearance of bacillary growth in inoculated mice. The inocula originated in skin biopsy specimens of patients and in mouse passage material; there were 347 inocula suitable for study. The rate of appearance of bacillary growth in inoculated mice confirmed the hypothesis that nonsolid bacilli are inert and nonviable and that all growth originates from the solidly staining organisms. The minimal infectious dose was determined in four suspensions and was found to range from 40 to 3 solidly staining bacilli.—(From Trop. Dis. Bull.)

- ✓ Nakamura, M. Morphological changes in bacterial forms of *M. lepraemurium* caused by physico-chemical treatments. La Lepro 39 (1970) 257-262. (In Japanese, English summary)

The English summary appended to the paper is as follows. A relationship between Morphological Index of live and dead *M. lepraemurium* was studied by using light and electron microscopes. First of all, it was clarified that the most important factor for staining conditions influencing Morphological Index of acid-fast bacilli, especially *M. lepraemurium*, is the heating when stained. In particular, the Morphological Index of *M. lepraemurium* obtained by a staining method at room temperature was significantly changed by the method of staining with heat: by the latter, most of nonsolid *M. lepraemurium* were changed to solid acid-fast bacilli. Next, a comparison was made between Morphological Index of live *M. lepraemurium* and dead ones that were killed by artificial treatments. *M. lepraemurium* was inactivated by treatments with ultraviolet-irradiation, heating at 60°C for 30 min., chloroform, ether, toluene, and n-butyl-alcohol, whereas it was not affected by treatments with desoxycholate and petroleum ether. There were no differences between staining qualities of live and dead *M. lepraemurium*. However, the cell wall

destructions were illustrated in dead bacilli thus inactivated by artificial treatments, when observed by an electron microscope.—(Adapted from Trop. Dis. Bull.)

- ✓ Nakamura, M. Elongation of *M. lepraemurium* *in vitro* observed by using silicon-coated slide glasses. La Lepro 39 (1970) 253-256. (In Japanese, English summary)

Elongation phenomena of *M. lepraemurium* *in vitro* were observed using silicon-coated slide glasses compared to common slides. The results obtained indicate that *M. lepraemurium* promptly shortened after being smeared on the silicon slide glasses and that remarkable elongation of *M. lepraemurium* on the silicon slide was observed only in the medium which was the most appropriate for elongation of the bacilli studied by using common slides. It could be noted from the results mentioned above that these findings might demonstrate that the bacilli fixed more faster on the silicon slide than on the common slide. On the other hand, it could be said that subtle morphological changes in the bacilli during cultivation might be missed if the silicon slide was used for observation of the bacilli.—Author's Summary

- ✓ Nakamura, M. and Kanagawa, S. Comparisons of growth curve and generation time of four strains of *M. lepraemurium* in foot pads of mouse CF No. 1. La Lepro 40 (1971) 143-145. (In Japanese, English summary)

Growth curves and generation times of four strains of *M. lepraemurium* were compared with the inoculation method of foot pad of mouse CF No. 1 strain.

The results obtained indicate that the generation time of the Kurume No. 42 and the Kumamoto strains had two steps of growth curve; in the case of the Kurume No. 42 strain, the generation time in the first log phase was 2.2 days and that of second one was 2.5 days. In the case of the Kumamoto strain, the first was 3.0 and the second was 4.5 days, respectively. On the other hand, the Hawaiian and Fukuoka No. 1 strains grew smoothly with one log

phase; these generation times were 6.6 and 13.6 days, respectively.—(Adapted from Trop. Dis. Bull.)

Nakamura, M. and Kanagawa, S. Growth curves and generation times of Hawaiian strain of *M. lepraemurium* in foot pads of three strains of the mouse. La Lepro 40 (1971) 146-148. (In Japanese, English summary)

The Hawaiian strain of *M. lepraemurium* was individually inoculated into the foot pads of CF No. 1, dd, and C₃H strains of the mouse and the growth curves and generation times were estimated. The generation time of the bacilli was 3.3-4.2 days in the case of CF No. 1 mouse, 5.1-8.5 days in the dd mouse, and 2.9-3.3 days in the C₃H mouse, respectively.—(Adapted from Trop. Dis. Bull.)

Nakamura, M. and Kanagawa, S. Growth curves and generation times of four strains of *Mycobacterium lepraemurium* in the foot pad of the dd and C₃H strains of mice. La Lepro 40 (1971) 179-183. (In Japanese, English summary)

Comparisons of growth curves and generation times of four strains of *M. le-*

praemurium (Kurume No. 42, Hawaii, Kumamoto and Kukuoka No. 1), inoculated into the foot pads of the dd and C₃H strains of mice were made and the results obtained as follows: one-step growth curve was observed in the foot pad of the dd mouse in the case of all strains of *M. lepraemurium* tested; on the other hand, in the case of the C₃H mouse, one-step growth curve of Kumamoto and Hawaiian strains, and the two-step growth curve of Kurume No. 42 and Fukuoka No. 1 strains, were observed.

The Kurume No. 42 and Kukuoka No. 1 strains had approximately four days of generation time. The Hawaiian and Kumamoto strains had six to eight days and four days respectively, when the bacilli were inoculated into the foot pad of the dd mouse. When the bacilli of the Hawaiian and Kumamoto strains were inoculated into the C₃H mouse foot pad, the generation time of the former was approximately four days, ten days for the latter. In conclusion, there might be two types of *M. lepraemurium*; *M. lepraemurium* whose growth should be modified by the strain of mice employed, and the other would have a characteristic growth rate which should not be influenced by the type of mice used.—(Adapted from authors' summary)

Experimental Infection

Fieldsteel, A. H. and McIntosh, A. H. Effect of neonatal thymectomy and antithymocytic serum on susceptibility of rats to *Mycobacterium leprae*. Proc. Soc. Exp. Biol. Med. 138 (1971) 408-413.

Neonatal thymectomy and neonatal thymectomy plus ATS resulted in a marked increase in susceptibility of both Buffalo and Lewis rats to infection with *M. leprae*. In Buffalo rats, increase in susceptibility was limited to foot pad infection. In Lewis rats, this was extended to include testis infection where the organisms in thymectomized-ATS-treated rats were found to be still in the logarithmic phase of growth 15 months after inoculation.—Authors' Summary

Glasgow, L. A. and Bullock, W. E. Impairment of interferon production in mice infected with *Mycobacterium lepraemurium*. Exp. Biol. Med. 139 (1972) 492.

Mice infected with the Hawaiian strain of *M. lepraemurium* were tested for interferon levels in five experiments, after being inoculated intraperitoneally with representative inducing agents including viruses and bacteria. An 85% reduction was observed at the time of the peak interferon response. No alterations in time relationships of response were found. The effect of *M. lepraemurium* on the capacity to release "p formed" interferon showed consistent stimulation of release at 2- to 4- fold greater

levels of interferon in the serums of infected animals.—G. L. Fite

Goihman-Yahr, M., Avila, J. L. and Convit, J. The methylene blue test in experimental granulomas. *J. Invest. Derm.* **58** (1972) 74-77.

Experimental granulomas were induced in lepromatous volunteers, rabbits and guinea pigs, by the intradermal injection of incomplete Freund's adjuvant.

Experimental hypercholesterolemic xanthomatosis was produced in rabbits by cholesterol feeding.

Despite histological and histochemical similarities between such lesions and lepromata, and the fact that chloroform-methanol extracts of experimental lesions bound methylene blue, no coloring could be seen after parenteral administration of this dye at doses far above those needed to stain lepromata.

The blueing phenomenon then, is one of relative specificity. We suggest that it is due to some metabolic derangement induced by *Mycobacterium leprae* in a susceptible parasitized macrophage. This might involve oxidation-reduction processes and/or some unusual lipid from cells or bacilli, that were unable to bind methylene blue in a stable complex.—Authors' Summary

✓ **Kawaguchi, Y.** Classification of experimental mouse leprosy—with special reference to intermediate type. *La Lepro* **40** (1971) 16-22.

As stated in our previous reports, comparative observations were made on the development of experimental murine leprosy in various inbred strains of mice concerning the relationship between subcutaneous leproma at the inoculation site and evolution of visceral lesions. On the basis of the findings in these experiments, this author considered that mouse leprosy could be classified into two polar types, benign and malignant. In the former type which is represented by C57BL/6 and ddY strains, the subcutaneous leproma appears earlier and is small, hard and sharply defined, whereas the leproma of the latter type,

which is represented by C3H strain, develops slower but becomes much larger and soft with a diffused thickening appearance. In the case of the malignant type, visceral lesions became pronounced with time, this extensive involvement could have a fatal outcome. On the other hand, the visceral organs of the benign type revealed, in general, the presence of slight lesions even in the advanced cases.

However, it was obvious from the results of long-term observation up to 80 weeks that, in the cases of CF#1 mice, subcutaneous infection with murine leprosy bacilli were found to give a disparity in the relationship between the feature of leproma at the inoculation site and the involvement of visceral organs: i.e., subcutaneous leproma showed the benign feature but visceral lesions became as prominent as observed in the malignant cases.

To solve this disparity, further long-term observations up to 80 weeks were made in various inbred strains of mice (C3H, fm, CFW, BALB/C, KK, DDD and C57BL/6) by the same manner as described above.

In strain C3H, the typical malignant course of the disease was followed throughout the observation period. The disease course of fm mice was compared to that of C3H mice. In the mice of C57BL/6 and DDD strains, subcutaneous lepromas showed the benign type and remained generally small with a tendency to regress at the late stage of infection. At the end of experiment two C57BL/6 mice were extremely emaciated and died, showing pronounced visceral lesions. But in the other mice of these two strains, no extensive involvement was observed in their organs.

On the contrary, in mice of KK, BALB/C and CFW strains, the feature of subcutaneous leproma at the inoculation site was typical benign, but the development of visceral lesions was comparable to that of malignant cases.

Taking these facts into consideration, it would be reasonable to assume that the disparity mentioned above might result from the grade of developing immunity in the hosts. Mouse leprosy should be then classified into three types; benign, intermediate and malignant. In the case of in-

intermediate type, newly classified, the leproma at the inoculation site shows the benign feature but the visceral lesion becomes pronounced with time so as to resemble those in the malignant cases.—(Adapted from author's summary)

Nakamura, K. and Hisai, S. [Experimental inoculation of *M. leprae* into the foot pads of Asiatic chipmunks (*Tamias sibiricus*).] La Lepro 39 (1970) 205-209. (In Japanese, English summary)

The bacilli multiplied, but to a lesser extent than in mice. Nodular swelling occurred in the foot pads.—(From Trop. Dis. Bull.)

Nakamura, K. and Hisai, S. Multiplication of *M. lepraemurium* in the foot pads of mice and hamsters. A basic study of inoculation and the effect of thymectomy or thymectomy plus the administration of immunosuppressive agents. La Lepro 39 (1970) 7-16. (In Japanese, English summary)

This study was carried out to examine the minimum inoculum, the differences in the strain, sex and age of mice, and the multiplication of *M. lepraemurium* in the hind foot pads of mice and hamsters which had been subjected to thymectomy, thymectomy plus the administration of immunosuppressive agents, injection of Freund's incomplete adjuvant, and gamma-ray irradiation (approximately 1200 rad) in thymectomized mice.

The strains of mice used were ICR, SW/A, CF#1, ddY-F and dd, and the hamsters used were the golden hamster.

The results obtained are summarized as follows:

1) The minimum inoculum was approximately 5.0×10^2 bacilli per each hind foot pad.

2) Nodular swelling in the hind foot pad of the mice was occasionally greater in the male than in the female, but that of the hamsters was less in the male than in the female, as was true in thymectomized hamster. Also, the differences for the age of 4 weeks (adults) and that of 6-8 months (old) in the female mouse were less in the old than in the adult. Furthermore, no significant difference was observed between the CF#1 strain of mice and the others.

3) In the thymectomized mice and hamsters, leproma-formation in the hind foot pad was inhibited at an early stage after the inoculation, but accelerated at a later stage after the inoculation. This difference was apparently observed in the neonatally thymectomized mice. In addition, leproma-formation was strongly inhibited by the administration of anti-lymphocytic serum (ALS).

4) The effect of immunosuppressive agents on the multiplication of *M. lepraemurium* was apparent in testosterone propionate or progesterone-administration, but such other agents as cortisone, thyroxine, pituitary hormone, reserpine, hydroxyurea, mitomycin C, 5-fluorouracil and 6-mercaptopurine, did not show good results in our investigation. However, in the case of the hamsters leproma-formation was inhibited by the administration of antilymphocytic serum (ALS).

5) The count of *M. lepraemurium* and leproma-formation in the foot pads was strongly inhibited by an injection of an adjuvant and by gamma-irradiation.

It may be concluded from these results that the multiplication of *M. lepraemurium* in the foot pad of mice and hamsters was mainly influenced by the presence or absence of immunity, i.e., the depression of circulating lymphocyte levels in the granuloma formation.—(Adapted from authors' summary)

Epidemiology and Prevention

Blanc, F. and Nosny, Y. La lèpre et les lépreux à Marseille. [Leprosy and leprosy patients in Marseilles.] Bull. Soc. Path. Exot. 63 (1970) 417-429. (In French, English summary)

The authors review briefly the evidence that indigenous leprosy has all but disappeared from the center and south of France during the past century. There are probably well over 100 people in Marseilles and

its environs suffering from leprosy today, and virtually all have contracted the disease outside France. Some are under regular medical treatment; others probably remain undiagnosed or misdiagnosed; while others hide their signs and symptoms out of fear or shame. Those in the last two categories probably out-number those in the first category.

Out of the 100 patients diagnosed in their clinics, the authors give details of the 32 who were treated by them either as in-patients or at their consultations. Half were Europeans, all of whom had contracted the disease abroad, with one possible exception. No confirmed case of indigenously contracted leprosy has been seen by them. The latent period between exposure and the appearance of the first sign of leprosy can be put at between two and six years. Of the 16 European patients, 11 suffered from severe lepromatous leprosy, but only 3 of the 16 non-Europeans, but it is remarked that obvious lepromatous disease would be a bar to immigration. Misdiagnosis included polyneuritis of undetermined etiology, syringomyelia, allergic skin reactions, *erythema nodosum*, mycoses, actinomycosis, erysipelas. The authors comment on the widespread (but erroneous) belief that the lepromin reaction (Mitsuda) has diagnostic value.

After a summary review of treatment, the authors ask, and attempt to answer, the question of the possibility of leprosy becoming endemic in France. A few rare cases of proved indigenous leprosy around Bordeaux and Paris do not invalidate the contention that, despite close cohabitation, poverty and lack of hygiene, leprosy has failed to establish itself in and around Marseilles. Hence, ambulatory treatment, coupled with some effective public health control, should suffice to prevent any outbreak of leprosy in the conditions prevailing at present in France. They give a salutary warning that the reactional episodes occurring in the course of lepromatous leprosy are difficult to treat, and that in such patients definite cure is impossible to establish with certainty.—S. G. Browne (*From Trop. Dis. Bull.*)

Castellazzi, Z. Epidemiología de la lepra con especial referencia a Venezuela. [Epidemiology of leprosy with special reference to the position in Venezuela.] Rev. Venez. Sanid. Asist. Soc. 35 (1970) 429-480.

The author is in charge of the anti-leprosy campaign in Venezuela and in this paper gives a most extensive and instructive review of the present position of the disease in the world and, more particularly, in his own country. He mentions that the infecting organism can be found in practically every tissue of the body except the lungs, and even in the milk of infective mothers. He states that work is now proceeding in his laboratory to determine whether healthy carriers do in fact exist. The disease may be transmitted by arthropods *via* the skin. The author mentions that in two stages in Venezuela it has been shown that fleas (presumably *Pulex irritans*, but not stated) have been found infected. While the incubation period may range from a few months to over 40 years, he states that two-thirds of children exposed to the infection acquire it before their second birthday. The host-bacillus relationship determines the various types of the disease as exemplified by the highly infectious lepromatous form on the one hand and the slightly infectious tuberculoid form on the other, with intermediate (borderline) forms (dimorphic and indeterminate) being unstable. From the epidemiological point of view the lepromatous form is not likely to be due to an especially virulent organism. A detailed description is given of the Mitsuda (lepromin) reaction, its various stages and reliability, and the author mentions that an early reaction (3 or 4 + in 24-28 hours) agrees in over 90% of cases with the true Mitsuda. In his experience, all those with reactions smaller than 6 mm can be regarded as negative and the remainder as positive, but the size of reactions increases with the age of the person tested, while a high percentage of persons living in areas where leprosy is unknown react; BCG tends to make the Mitsuda test positive. The author discusses the factor N hypothesis, but states that the presence of antibodies is of absolutely no

value as an indicator of any defense mechanism.

A description is given of the history of the disease in Venezuela, to which it was apparently brought by Norwegian sailors. The first known case was that of the governor of the country in 1626; by 1760 a hospital for leprosy sufferers had been set up. Since then, various decrees have been published, and the Leprosy Division of the Venezuelan National Health Service was set up in 1946. From then to 1968 the number of patients notified was 17,359; 2,183 were cured, 1,859 died and 8,238 remained under control and treatment. In 1968, of the 588 cases notified, over 40% were of the highly infectious lepromatous or dimorphic types. The condition is by no means found only in rural areas, and the migration from these areas to the towns and cities makes it a serious problem for the public health service as, while the incidence for the whole country is 1 per 1,000; in Apure State (in the southwest of the country) it is as high as 6 per 1,000. In 1968, of 3,658 contacts, 1,689 lived with infected persons and 1,147 of these contacts were under the age of 15 years. Thirty-seven new cases were diagnosed in children under the age of 10 years and a further 32 in those between 10 and 15 years, and of these 69 patients 8 had either the lepromatous or the dimorphic form. The highest incidence occurs in adults. The author ascribes an apparent increase in the incidence of leprosy in persons over the age of 60 years to a lessening of the natural defense mechanism; the sex ratio is 2 males to 1 female. Neither climate nor the various physiological conditions, such as puberty, pregnancy, and the menopause, seem to have much to do with the incidence of the disease. With regard to diagnosis, the importance of epidemiology, and clinical, bacteriological and histological examinations, is stressed. Finally, the author once again emphasizes that the intermediate forms are unstable.—W. K. Dunscombe (*From Trop. Dis. Bull.*)

✓ Galicia, R. H. Vigilancia epidemiológica de la lepra en México. [Epidemiological surveillance of leprosy in Mexico.] Bol.

Ofic. Sanit. Panamer. 69 (1970) 229-237. (In Spanish, English summary)

The author first gives an interesting historical account of leprosy in Mexico. He states that the disease was first brought to the country in 1521 by the "conquistadores," then in the 16th and 17th centuries it entered from the west coast with the coming of Filipino sailors. At the same time it entered Europe. The focus in the northeast of Mexico arose due to immigration from the infected central part of the country.

Measures of control began as early as 1528, when Cortés ordered the construction of a hospital for leprosy patients; this stage lasted till 1927 when regulations were first made by the federal government and a census of patients was taken. Between 1927 and 1948, six leprosaria were built and the seclusion of patients was ordered. In 1948 treatment by the sulphones began. This stage lasted till 1960 when the present program of ascertainment, treatment and detection of contacts by teams of doctors and nurses started. Contrasts in the number of cases and the rate of infection per thousand, in the 31 states and the Federal District, in 1946 and 1968, are shown in a table. In 1946 the State of Colima had a rate of 3.27 per 1,000, two other states had rates of 1.37, and a total of 8,177 cases were registered. In 1968, 14,463 cases were registered, but no state had a rate of more than 1.46.

In 1930-1946, there were three main large foci which were still in existence in 1968, but to a greatly reduced extent. In 1968 there were 8,082 lepromatous, 3,069 tuberculoid, 3,283 indeterminate and 29 unclassified cases; 8,672 cases occurred in males; only 4.8% of the patients were under the age of 15 years. It is thought that the incidence in younger persons is greater than suspected hitherto. Stress is laid on the importance of detecting contacts and their careful supervision.—W. K. Dunscombe (*From Trop. Dis. Bull.*)

✓ Hausfeld, R. G. An anthropological method for measuring exposure to leprosy in a leprosy-endemic population at Karimuri, New Guinea. Bull. WHO 43 (1970) 863-877.

The author describes the method he has developed and which he has applied in the field to assess in comparable form the degree to which people were exposed to others suffering from potentially infective leprosy. The population concerned was the isolated tribe in a mountain valley in New Guinea which has been subject to a controlled trial of the value of BCG vaccination in preventing overt signs of leprosy infection (Scott *et al.* Trop. Dis. Bull. 64 [1967] 376.

The method essentially consists of identifying the various possible kinds of contact both within and outside the household of residence. Scores are given to the levels of intensity of the contacts, and the results are so recorded that they can be fed into a computer for analysis and comparison.

An early practical result of the application of this study was the confirmation of the randomness of the selection of individuals for BCG vaccination. Various hypotheses are currently being tested, especially the contagiousness of lepromatous leprosy and the kind of exposure that precedes the manifestation of the disease in contacts. (Other relevant factors of epidemiological

importance are doubtless being considered, such as the dissemination of viable bacilli by putative index cases, and the individual differences shown, for example, by sibs in the closeness and duration of their contacts with parents.)—S. G. Browne (*From Trop. Dis. Bull.*)

Mali, I. B. Leprosy in Nepal. J. Nepal Med. Ass. 9 (1971) 31-36.

A WHO leprosy survey in Nepal in 1963 indicated an incidence of leprosy in the range of ten cases per thousand. The author gives added figures on selected areas suggesting that the prevalence is relatively stationary. Two leprosaria in Nepal are described as of the style of a century ago. One houses 562 patients. The author stresses the need for outpatient services for patients, provided by general hospitals, with a continued search for cases in early stages of the disease. Leprosy laws of Nepal need modification, and if patients are to be kept active and deformities prevented, better medical services need to be made available.—G. L. Fite

Other Mycobacterial Diseases

Armstrong, J. A. and Hart, P. D. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. J. Exp. Med. 134 (1971) 713-740.

The cytological response to the ingestion of tubercle bacilli by cultured mouse peritoneal macrophages has been studied by electron microscopy. In the bacterial suspensions used routinely for inoculation, about half the bacilli were viable, matching closely the proportions of intact and damaged organisms identified with the electron microscope. It is inferred that intracellular survival of *M. tuberculosis* in cultured macrophages is associated with a tendency to nonfusion to dense granules with the phagosome, thus avoiding direct exposure of the bacilli to the contents of these organelles. It is suggested further, that fusion

of dense granules with the phagosome leading to digestion is determined by recognition of the bacillus as nonviable. The possibility is discussed that the cytological response to different mycobacterial infections may reflect differences of a basic nature between facultative and obligate intracellular parasitism. [This detailed article would be valuable reading to anyone interested in a parallel investigation using *M. leprae*.]—G. L. Fite (*From authors' summary*)

Chang Yong Cha, Dong Soon Kim and Seung Hoon Lee. A study on the macrophage-mycobacterium interactions *in vitro*. Tubercul. Res. Dis. 18 (1971) 12-20.

Numerous studies on the interactions between phagocytes and pathogens have been reported; however, information on the

biology of the pathogen in the host, or the biochemical alterations occurring in the tissue of the host due to infection is incomplete or missing. It would be worthwhile to learn more about the mechanisms involved in the maintenance or destruction of function and life as a consequence of the relationship between phagocytes and pathogens.

Thus, an experiment was planned and performed to study the mode of interactions between rabbit macrophages and mycobacteria, which are facultative intracellular parasites, at different temperatures which might influence the metabolic activities at various cells.

Normal rabbit macrophages were collected from the peritoneal cavity, suspended in the CMF medium and mixed with suspensions of mycobacteria. The macrophage-bacteria suspensions were maintained for 24 hours at 37°C and 33°C. The rate of appearance of the macrophages containing the organisms and the extracellular and intracellular viable population of the organisms were studied.

The results of the observation were summarized as follows:

1. At 37°C and 33°C percentages of the intracellular units to the total viable units of *Mycobacterium tuberculosis* (H₃₇Rv) reached 63% after 1 hour and after 6 hours above 65%. Percentages of macrophages containing H₃₇Rv strains began to show above 40% after 2 hours at 37°C and 33°C.

2. Percentages of the intracellular units to the total viable units of *Mycobacterium bovis* (Ravenel) reached 61% at 37°C, or

54% at 33°C after 1 hour, but after 3 hours 70% were phagocytized at both temperatures. After 2-3 hours 40% of macrophages contained Ravenel strains in the cytoplasm at 37°C and 33°C.

3. Percentages of the intracellular units to the total units of *Mycobacterium bovis* (BCG) showed 45% after 1 hour, and 60% after 3 hours at 37°C and 33°C. After 3-6 hours, 40% of macrophages contained BCG strains intracellularly at 37°C and 33°C.

4. Percentages of the intracellular units to the total units of *Mycobacterium ulcerans* showed 42% at 37°C, or 47% at 33°C after 1 hour, and after 3 hours 56% at 37°C or 60% at 33°C. Percentages of macrophages containing *M. ulcerans* were 32% at 37°C or 42% at 33°C after 3-6 hours.

5. 40% of the total viable units of *Mycobacterium fortuitum* were phagocytized after 1 hour, and about 50% after 6 hours at 37°C and 33°C. At both temperatures, 48% of macrophages contained *M. fortuitum*, within cytoplasm after 3-6 hours.—(From Korean Med. Abstr.)

Hepper, N. G. G., Karlson, A. G., Leary, F. J. and Soule, E. H. Genitourinary infection due to *Mycobacterium kansasii*. Mayo Clin. Proc. 46 (1971) 387-390.

This is essentially a case report of an infection involving the testis and adnexae. Bacilli were found in the urine and some prostatic involvement may have been present. Prolonged treatment with INH, streptomycin, and another drug, for three years was indicated, although the third drug was not clearly stated.—G. L. Fite