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EDITORIALS

Editorials are written by members of the Editorial Board, and opinions expressed are those of the writers. Any statement that does not meet with agreement will be of service if it but stimulates discussion, for which provision is made elsewhere.

THE IMMUNOLOGY PROBLEM 8

Work in connection with the immunology of leprosy has been pursued from two viewpoints. One is that of the laboratory worker, searching for a means of specific identification of the causative germ of the disease, with relation to micro-organisms cultivated from patients. The other is that of the clinical worker who seeks a method of specific diagnosis of cases. This second aspect of the problem is of most concern to the majority of leprosy workers, who are in need of such an aid in the diagnosis of early and doubtful cases and a means of detecting infected persons before the appearance of symptoms.

With increasing emphasis on work in the epidemiological field, and with at the same time extension of the basis of clinical diagnosis of the disease at least presumptive to characters less certain of identification than the familiar and unequivocal "cardinal" signs, the need of a reasonably sensitive and dependable immunological test is felt more than ever before. As yet we seem far from the desired accomplishment; in fact, with disappointments that have been met along certain lines of approach that seemed possibly hopeful until they were explored, the prospect is rather discouraging.

Considering the findings of many investigators in leprosy,

and experience in the analogous infection, tuberculosis—an analogy which of course must be used with caution and reservation—there seems to be no hope whatever for aid from nonspecific procedures that depend upon chemico-physical changes in the blood constituents. Nor is much that encourages hope from tests that depend upon the presence of specific antibodies in the blood. The field of allergy, referring particularly to tests that depend upon variable reactivity of the skin, appears to be the most hopeful one, but here we can say little more than that the field of search has been narrowed.

With reference to the nonspecific tests, the various reactions that depend upon quantitative and qualitative changes in the serum proteins, mostly devised for use in syphilis, seem utterly without promise. Nothing in recent recorded work indicates a modification of this conclusion which the writer reached after working with the Klausner's distilled water precipitation test, Bruck's nitric acid precipitation test, the formol gel test, and Naegeli's viscometric-refractometric method of (supposedly) determining the albumin-globulin ratio of blood serum, which were investigated in parallel with the erythrocyte sedimentation rate.¹ In this general category, so far as leprosy is concerned, are the several flocculation tests, for example that of Vernes, with which numerous workers have experimented.

The reaction devised by Rubino, sedimentation of formalinized sheeps' red-cells, has received considerable attention.² Adant concluded that it would be useful in diagnosis but other writers have shown it to be insensitive. The highest figures on positive findings published, those of Imbert, give only 62 percent in neural cases. Bier and Arnold got only 29 percent, with 14 percent in "incipients." Lépine, Markianos and Pupyoyannow, working with sera of Greek lepers, and Besta and Marini in Italy, concluded that it does not become positive until the clinical diagnosis is evident and that it has no value for diagnosis or prog-

¹ RAO, G. R. *Indian Jour. Med. Res.* 19 (1932) 993. GILLIER, R. *Bull. Soc. Path. exot.* 27 (1934) 707. LEFROU, G. *Ibid.* 29 (1936) 394. WADE, H. W. *Trans. 6th Cong. Far Eastern Assn. Trop. Med., Tokyo, 1925.* Tokyo, 1926, vol. 2, pp. 637; *Philippine Jour. Sci.* 30 (1926) 59.

² RUBINO, M. C. *Rev. Méd. Uruguay* 29 (1926) 143; *Rev. Soc. Argentina Biol.* 2 (1926) 407; *Compt. rend. Soc. Biol.* 96 (1927) 225; *Ann. Inst. Pasteur* 47 (1931) 147; etc. ADANT, M. *Ann. Soc. Belge Méd. trop.* 12 (1932) No. 4. IMBERT, M. F. P. *Puerto Rico Jour. Publ. Health & Trop. Med.* 12 (1936) 257. BIER, O. G. AND ARNOLD, K. *Ann. Paulista Med. & Cir.* 29 (1935) 177. LÉPINE, P., MARKIANOS, J. AND PAPAYOANNOU, A. *Bull. Soc. Path. exot.* 25 (1932) 543. BESTA, B. AND MARIANI, G. *Gior. Italiano Mal. Exot. Trop.* 9 (1936) 11. RUBINO, M. C. *Rev. Argentina Dermatof.* 18 (1934) 147.

nosis. Rubino himself only claims specificity for it, and not high sensitivity. However interesting this reaction may be academically on account of its apparent specificity, it does not appear to be of much value in practical work.

Certain tests devised for the diagnosis of cancer have been applied to leprosy, with interesting but as yet unevaluated results. Marras³ employed the principle of Ascoli and Izar's "miostagmin" test, by which the surface tension of the serum-antigen mixture is lowered, using a synthetic antigen of ricinolic acid. He reported it to be useful, positive even in "initial" cases, but nothing more has been heard of it. A modification of Fuch's carcinoma reaction, which involves the dermination of residual nitrogen, was employed by Minami, Hikichi and Hayata.⁴ It is claimed to be the best laboratory test for leprosy, always positive and especially valuable in the early stages, and at least as sensitive and specific as the Wassermann reaction in syphilis, though it does not appear that it has been applied to doubtful cases or contacts. Kawasaki obtained 95 percent positive reactions—93 percent in neural cases. Ikei and Huzimoto both investigated it in rat leprosy, the latter concluding that frequently the diagnosis cannot be established by the reaction at the beginning of the infection.

Turning to the specific serological reactions, the agglutination test seems to be definitely out. Weak and cross (group) reactions, and other difficulties make it of little value even in laboratory experimentation with acid-fast bacteria. Rather more hope has been held for the precipitin and complement-fixation reactions because of the possibility of eliminating from the test antigens the elements responsible for nonspecific reactions, and extensive work has been done recently to isolate the specific elements of mycobacteria. In tuberculosis the precipitin test has certainly not attained any important role as yet. It has recently been used extensively by Henderson⁵ in a study of the relationships of a large group of acid-fast bacteria, including many isolated from leprosy, but its potentialities with any of the available antigens for the diagnosis of that disease are not yet known. Even with a specific antigen in hand, it would be a question whether in very early or slight infections

³ MARRAS, A. *Biochem a Terap. Exper.* 15 (1928) 264.

⁴ MINAMI, S., HIKIZI, K., AND HAYATA, H. *Hifuto-Hitsunyo* 2 (1934) 9. HAYATA, H. *La Lepro* 5 (1934) 33. KAWASAKI, S. *La Lepro* 8 (1937) 27 and 65. IKEI, S. *Ibid.* 7 (1936) 1, and 8 (1937) 17. HUZIMOTO, S. *Ibid.* 8 (1937) 25. MINAMI, S., KAWASAKI, S. AND HUZIMOTO, S. *Japanese Jour. Dermatol. & Urol.* 42 (1937) 37.

⁵ HENDERSON, H. J. *THE JOURNAL* 5 (1937) 267.

there would be a sufficient concentration of antibodies in the blood to make this test of service.

Similar considerations apply to the complement fixation test. In tuberculosis much work has been done with it, and it is used in some places, but its value seems to be confirmatory rather than primarily diagnostic; it cannot be depended upon to give positive results in early or obscure cases in which clinical and bacteriological methods do not suffice for diagnosis. In leprosy numerous investigators have used it with various antigens, some of them of strange nature, but only the more recent work with antigens derived from cultivated micro-organisms need be mentioned here.

Ota and Ishibashi⁶ employed several mycobacteria in this way and found that one of the leprosy strains of Ota and Sato gave an antigen that was specific for leprosy. As usual, these investigators used sera from established cases, not incipient ones or suspects. The so-called *Streptothrix leproides* (Deyke), defatted, was used by Gomes, whose method was tried by de Assumpção and da Silveiro.⁷ Gomes found that almost all cutaneous-type cases but only two-thirds of the neural ones gave positive reactions, while "incipient" or "frustrated" cases were usually negative; on the other hand, rather high percentages of persons without clinical evidence of infection gave positive reactions. De Assumpção and da Silveiro, having applied the test comparatively, with controls, concluded that it is not specific and that a positive reaction is only suggestive of leprosy. High claims have been made for the tuberculosis antigen devised by Witebsky, Klingenstein and Kuhn.⁸ Pereira recommends that it be always used in cases difficult of diagnosis and to detect latent leprosy, but 32 percent of 84 contacts that he tested gave positive reactions; one-half of those persons showed no suspicious lesions, while some that had such lesions were negative. Lleras Acosta⁹ has reported especially interesting results with an antigen prepared from an organism which he has cultivated from leprosy. His findings

⁶ OTA, M. AND ISHIBASHI, T. THE JOURNAL 2 (1934) 413; *Japanese Jour. Dermatol. & Urol.* 37 (1935) 64.

⁷ GOMES, J. M. THE JOURNAL 2 (1934) 265, and 3 (1935) 283. DE ASSUMPÇÃO, L. AND DA SILVEIRO, G. F. *Rev. Leprol. São Paulo* 2 (1935) 13.

⁸ BRANTS, J. *Dermatol. Wchnschr.* 93 (1932) 1688. AOKI, J. AND MURAO, K. *Zeitschr. f. Immunitätsf.* 79 (1933) 365. PEREIRA, P. C. R. THE JOURNAL 4 (1936) 207. BIER, O. G. AND ARNOLD, K. *Arch. f. Schiffsu. Trop.-Hyg.* 39 (1935) 238. RABELLO, JR. AND MACHADO, J. C. *Rev. Brasileira Leprol.* 4 (1936) 307.

⁹ LLERAS ACOSTA, F. *Rev. Fac. Med. (Colombia)* (1936) 1-98, 99-199; Summary reprinted in THE JOURNAL 6 (1938) 91.

(reprinted in summary elsewhere in this issue) indicate a high degree of specificity and considerable sensitiveness. It seems desirable that his test should be investigated thoroughly.

In view of the situation thus outlined, and with thought to that in tuberculosis, hope has been placed mainly upon the development of a skin reaction analogous to the tuberculin test. Aside from the histamin test introduced by Rodriguez and Plantilla,¹⁰ which is not an immunological one and is necessarily of limited usefulness, there have been two main lines of approach, namely the "leprolin" reaction and tests made with extracts of cultivated acid-fast bacteria.

The leprolin test, in which a suspension of boiled leproma is used, has proved to be most interesting.¹¹ Used first by Mitsuda more than twenty years ago, it was not given much attention until after it was taken up by Bargehr in 1926. Hayashi, who worked with Mitsuda, summarized briefly their findings and showed results of parallel injections of various acid-fast bacilli from cultures. Chi-yuto and Manalang have based sweeping conclusions regarding infectibility on the differences of reaction on the part of very young children and older persons. Muir experimented with the reaction and made parallel tests with suspensions of rat leprosy leproma. The most recent study of it is that of Rodriguez which appears elsewhere in this issue.

Interesting as this reaction is, and valuable as it may be in connection with classification and prognosis—that is, in determining the cutaneous reactivity of patients, both in progressive phases of the disease and during recovery—it is obviously not a diagnostic test. Normal persons above a certain very early age, and infected persons with benign (neural-type) leprosy, alike give positive reactions as a rule, while the more seriously affected patients, with the malignant (cutaneous-type) of the disease, are usually negative. Furthermore, a positive reaction does not appear promptly; it is not looked for in less than a week and often does not reach the maximum for three weeks. These differences in behavior from that of the tuberculin reaction evidence an essential difference as

¹⁰ RODRIGUEZ, J. AND PLANTILLA, F. C. *Philippine Jour. Sci.* 45 (1931) 123; reprinted in *THE JOURNAL* I (1933) 49.

¹¹ MITSUDA, K. *Japanese Jour. Dermatol. & Urol.* (1916), cited by Hayashi; *Trans. 3rd Internat. Lep. Conf., Strasbourg, 1923.* BARGEHR, P. *Zeitschr. f. Immun. u. Exper. Ther.* 47 (1926) 529, and 49 (1926) 346. HAYASHI, F. *THE JOURNAL* I (1933) 31. CHIYUTO, S. *Month. Bull. Philippine Health Serv.* 12 (1932) 300. MANALANG, C. *Ibid.* 12 (1932) 308. MUIR, E. *Lep. in India* 5 (1933) 204; etc. RODRIGUEZ, J. N. *THE JOURNAL* 6 (1938) 11.

regards the factors or process involved. It would seem (as noted by Rodriguez¹²) that the elements that are directly concerned in the production of the reaction lesion may not be formed until after the antigen is introduced. In other words, the test appears to be one of capability to react to the presence of the killed bacilli and not of the actual existence of specific hypersensitiveness, or allergy. Be that as it may, there is no indication that the test is of any value for the purposes for which a sensitive, specific test is so badly needed.

The remaining line of approach, skin reactions with purified extracts of acid-fast bacteria, has recently been shown quite conclusively to be without hope so far as the available races of those organisms are concerned. Henderson, Aronson and Long¹³ tested 158 patients with highly purified proteins of two strains cultivated from lepers, but they concluded that the reactions did not show sufficient specificity for practical diagnostic use. To investigate the matter thoroughly McKinley recently collected and brought to the Philippines no less than twelve protein and five other antigens; antigens, nine of these proteins and three other antigens (a polysaccharide, leprosin and leprosinic acid) had been obtained from mycobacteria isolated from human leprosy. A report of the more than 5,000 skin tests made on a large number of persons of various categories appears in the present issue.¹⁴ None of the antigens used gave indication of being useful as a specific diagnostic reagent.

For the present the situation seems to be one of stalemate. If there is today any test or reaction which can be used to distinguish among suspects those persons who have leprosy from those who do not, or to identify infected persons who show no clinical symptoms of the disease, that fact has not been established. If, from the fact that preparations of the tubercle bacillus give specific and sensitive skin reactions in tuberculosis, it is permissible to hold the hope that similar preparations of the true leprosy bacillus may be similarly useful in leprosy, the situation emphasizes the importance of obtaining satisfactory quantity cultures of that organism. It is possible that a specialist in immunology might be able, by biochemical technique, to obtain a test substance from materials from patients, and that avenue should not remain unexplored. But even that approach would undoubtedly involve efforts to obtain purified bacterial substances.

¹² RODRIGUEZ, J. N. Personal communication.

¹³ HENDERSON, H. J., ARONSON, J. AND LONG, E. R. *Jour. Bact.* 31 (1936) 41.

¹⁴ MCKINLEY, E. B. *THE JOURNAL* 6 (1938) 33.

An effort now under way to obtain large quantities of bacilli in cultures has been described by McKinley and de Leon.¹⁵ It would be out of place here to discuss the identity of the organism in question, which is the one cultivated by Soule and McKinley in Puerto Rico in 1931¹⁶ and recultivated in the Philippines by Soule in 1933, but which has not yet adapted itself to saprophytic life sufficiently to permit doing more than maintain the strain. Whether or not it is the true leprosy bacillus in the form in which it occurs in the tissues, probably no one would care to say that it is humanly impossible to arrive at conditions of environment and nutrition that will permit satisfactory cultivation of the leprosy bacillus, in spite of its biological peculiarities. But as yet it remains a challenge to the bacteriologist. In the meantime the leprosy field worker awaits the production by the immunologist, whether from the cultivated organism or otherwise, of a test substance for use in practical diagnostic work.

¹⁵ MCKINLEY, E. B. AND DE LEON, W. *THE JOURNAL* 5 (1937) 259.

¹⁶ SOULE, M. H. AND MCKINLEY, E. B. *American Jour. Trop. Med.* 12 (1932) 1; reprinted in *THE JOURNAL* 1 (1933) 53. MCKINLEY, E. B. AND SOULE, M. H. *Jour. American Med. Assoc.* 98 (1932) 361.