INTRACELLULAR PARASITISM OF MYCOBACTERIA

SUTER'S REVIEW, AND HANKS' COMMENTARY

Recently observed facts of intracellular parasitism of the tubercle bacillus are arresting and intriguing for students of leprosy, whose causative agent is a mycobacterium peculiarly of intracellular habitus. Having encountered one of Dr. Emanuel Suter's reports of his studies in this field, and having examined other available literature,[1] we invited him to provide a review of the subject. The commentary by Dr. John Hanks is also a solicited contribution, complementing the other in more or less editorial fashion with special application to findings of the author and his associates in work much of which has been or will be reported in THE JOURNAL.

Although most of the studies of intracellular parasitism of bacteria have been recent and with tubercle bacilli, it appears that as long ago as 1916 it was demonstrated that typhoid bacilli are protected against chemicals and antiserum when located inside of intact cells. With the

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[1] Abstracts of most of these articles appear in the Current Literature section of this issue. In the present note, footnote references are not given for those reports which are repeatedly referred to elsewhere.

more chronic infection brucellosis, it has been shown that the organisms within phagocytes are largely protected against antibiotics and serum antibodies, making even combination treatments difficult.[2]

In the field of tuberculosis, Suter cites first the work of Barski with cultures of tissue from BCG-infected rabbits, and that of Jensen with *in vivo* treatment, which showed among other things that streptomycin cannot rid the tissues of the tubercle bacilli because the cells protect them. Evidently independently and more or less simultaneously, Suter in the United States and Mackaness in England demonstrated that virulent or attenuated (not avirulent) bacilli would grow in tissue-culture macrophages from normal animals—with more or less injury to the cells depending on the degree of virulence—even when the surrounding medium contained enough streptomycin to be bacteriostatic for extracellular bacilli. Isoniazid, on the other hand, was found to be as effective against intracellular bacilli in tissue cultures as against those in ordinary cultures.

The latter drug evidently enters the cells freely and exerts its full bacteriostatic activity there, whereas, as Barnett and Bushby [3] point out, streptomycin evidently has little power to penetrate the cells. They recall the opinion of Tzanck and Basset [4] that streptomycin is effective against leprosy bacilli only when they are extracellular and in the circulation (i.e., in reactional conditions), and that it is without effect on the organized lesions where the bacilli are intracellular and grouped in globi. They hold it as tacitly assumed that the sulfones, clinically active, effectively penetrate the cells.

Suter, in summarizing the first section of his review, says that "the most important lesson seems to be that combinations of chemotherapeutic agents are more potent and effective than any [single] agent alone. . . ." He also suggests that, in combinations, one of the drugs may perhaps be toxic for the infected cells and render them more permeable to the other drug, which then acts against the infecting agent.

Much has been heard, first and last, of combination treatments of leprosy, but how many clinical investigators have used any combination of useful or potentially useful drugs on a large enough scale and for sufficient lengths of time to arrive at any definite and well-founded conclusions about their effectiveness? The principal trials of which we are aware are those of the Clinical Evaluation Studies of the Leonard

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[2] HARRIS, H. J. Antibiotic and antigenic therapy of brucellosis with special reference to chronic disease; report of 421 cases. Antibiotics & Chemotherapy **5** (1953) 982; *abstract in J. American Med. Assoc.* **154** (1954) 447.

[3] BARNETT, M. and BUSHBY, S. R. M. The activity of iso-nicotinic acid hydrazide in murine leprosy. Lep. Rev. **24** (1953) 19-26; also, personal communication.

[4] TZANCK, A. and BASSET, A. La streptomycine dans le traitement de la lèpre. Bull. Soc. française Derm. et Syph. **57** (1950) 207-209.

Wood Memorial.[5-7] Besides single drugs, there were used dihydrostreptomycin plus diasone and dihydrostreptomycin plus PAS. No evidence had been seen, clinical or bacteriological, that either of these combinations was any better than the other treatments used. The treatment periods were only 32 and 48 weeks, in different units.

The second part of Suter's review deals with immunological findings that may have important implications for leprosy. First, however, he points out the main factors of virulence among tubercle bacilli: (*a*) the ability to multiply intracellularly (at least until the tissues develop immunity), and (*b*) the capacity of destroying the host cells. The leprosy bacillus has only the first of these characteristics; it does not injure those cells in which it is able to multiply.

As for the immunological features, whereas cells from normal animals permit tubercle bacilli to multiply in them under the conditions of the experiment, cells from animals "immunized" by BCG vaccination retard or completely inhibit intracellular multiplication.[8] This characteristic evidently depends on changes in the cells themselves, humoral factors having nothing to do with it, for it makes no difference whether the culture fluid contains normal or "immune" serum. As a matter of fact, the immune serum by itself is unable to prohibit multiplication of even extracellular bacilli.[9]

A question of interest is how this inhospitable attitude of the immune cells is acquired. Does it depend upon some complex reaction that can occur only in the animal body? Or can the macrophages which proliferate in tissue cultures in the presence of tubercle bacilli change, *in vitro*, from the normal to the immune state—i.e., acquire the lethal characteristic of epithelioid cells? No adequate answer to this question has been obtained.[10]

Suter suggests that the phenomenon of inhibition by immune cells is peculiar to tuberculosis, and that, like the agents of brucellosis and typhoid fever, the leprosy bacillus probably survives within phagocytes

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[5] DOULL, J. A. Clinical evaluation studies (first series), Leonard Wood Memorial (American Leprosy Foundation); preliminary report. I. Objective, organization and methods. Internat. J. Leprosy **21** (1953) 573-574 (abstract).

[6] DAVISON, A. R. Do. II. Clinical results. Ibid, p. 574 (abstract).

[7] GUINTO, R. S. Do. III. Bacteriological findings. Ibid, pp. 574-575 (abstract).

[8] This is a very different phenomenon from that which occurs when tuberculin is brought into contact with cells grown from sensitized animals. In that event it is the cells that are damaged, whereas in the present case the cells damage the bacilli apparently without suffering injury themselves.

[9] SUTER, E. Personal communication.

[10] BARSKI states that in cultures of tissues from infected animals—not in those from normal animals—"from the third to the fifth days there appear typical agglomerations of epithelioid tissue with very numerous giant cells of the Langhans type. . . . Only exceptionally do these cells contain acid-fast bacilli." This, however, is quite another matter.

"even after some form of immunity has been established." This is a point, we suggest, that is open to debate.

The leprosy bacillus exists unharmed and multiplies freely in the macrophages of cases of *lepromatous* leprosy, but there is no evidence that "immunity" in any usual sense of that term exists in such cases, only a remarkable "tolerance." On the contrary, definite lack of immunity is suggested by—apart from the free development of the bacilli—the nonreactivity of the skin to the bacillus suspension (lepromin)—a peculiarly specific nonreactivity, because it reacts positively to suspensions of other acid-fasts one may occasionally find a few bacilli in epithelioid cells, but activity in this form of the disease is evidenced by the "lepra reaction" conditions, but these phenomena do not bespeak immunity. They may, be held rather, to exemplify the existence of allergy without immunity.

We know something of what at least relative immunity in leprosy is like (although we can't explain it), from the maculoanesthetic and tuberculoid forms of the disease. Those forms, typically, are lepromin-positive, and in their lesions the monocytes and macrophages—and the epithelioid cells derived from them—do not permit free multiplication of the bacillus. One would be hard put to say precisely where and how the infection is maintained—whether or not it is outside of the phagocytes, as Suter suggests it may be in tuberculosis. In sections well-stained for acid-fasts one may occasionally find a few bacilli in epithelioid cells, but ordinarily such bacilli show evidence of degenerative changes and destruction, and the cells themselves may be pinkish from the diffused but undigested waxy element of the bacilli they have destroyed. The situation may of course be quite different, with more or less numerous bacilli present, when there is a reactional disturbance which lessens the antibacterial activity of the tissue cells and causes the condition to veer—however slightly and temporarily—toward the "borderline" state between typical tuberculoid and typical lepromatous.

As Hanks points out, he has observed significant differences in tissue cultures between cells (fibrocytes) grown from lesions of lepromatous and of tuberculoid cases. The latter kind rapidly reduced leprosy bacilli to acid-fast debris, although those which acquired numerous bacilli suffered injury and assumed epithelioid characteristics, including a rosy color after Ziehl-Neelsen. The fibrocytes from lepromatous cases, on the other hand, were indifferent to the presence of the bacilli even in large numbers. This important observation seems to have aroused little if any attention, and it may be taken as an indicator of the generally low status of laboratory research in leprosy that there has been no report of repetition for confirmation.

Hanks points out, further, that metabolic (enzymatic) limitations of the leprosy bacilli, and their susceptibility to unfavorable elements in the extracellular environment, appear to be the main factors leading to their

adoption of the intracellular habit. He suggests that *M. leprae* may be even less active metabolically (as well as more susceptible to inhibition) than the rat bacillus, and that because of this depressed basal metabolism the search for a dramatically effective drug for leprosy may be disappointing. This consideration, he holds, gives special interest to the immunological findings related by Suter. Like other mycobacteria, leprosy bacilli are undoubtedly vulnerable to the type of intracellular action which Suter has observed, and this should be given attention.

In closing this note we are impelled to quote the following from a personal communication from Dr. Hanks:

> It may be that not all leprologists will appreciate the full significance of Suter's review and whatever merit there may be in my comments on it. Because of the natural urge for better treatment, a large proportion of the available funds go into clinical work, much of which is misdirected for lack of guiding principles. At the present rates of investment in fundamental inquiry, there will continue to be a lag in basic information concerning the properties of the causative agent, the physiological situation that one seeks to modify by treatment, and the important advantage to be gained by successful immunological modification of cell response.

We heartily endorse these sentiments. The possibilities indicated by the articles of Suter and Hanks suggest that fundamental work in the microbiology of leprosy could be greatly intensified along modern lines with promise of profit to all concerned with this disease. For a beginning, we would suggest that in those leprosy institutions where serious laboratory work is being done, or could be done, the technique of study of macrophages in cultures should be acquired, and the reactions to the bacilli from lepromas on the part of cells from normal individuals and from patients with the different forms of leprosy should be studied, and also from patients vaccinated with BCG. But whatever line of microbiological investigation is undertaken, it would seem that closer cooperation between the laboratory investigator and the clinician is very much in order.

—H. W. WADE