INTRACELLULAR TUBERCLE BACILLI

In the field dealt with by Suter in a recent issue of THE JOURNAL[1] there seems to have arisen something of a controversy with Mackaness. The latter has reported findings at variance with some of those reported by Suter and by Lurie, and to explain the differences has offered criticisms of their methods or conclusions. They have registered disagreement with some of his views.

The first point—not the most important one to us—is whether or not the ability of tubercle bacilli to multiply in phagocytes *in vitro* is dependent upon or parallels their virulence. Suter[2] found that virulent and attenuated strains would grow equally well, whereas definitely avirulent strains would not multiply at all. He worked with guinea-pig monocytes. Mackaness and associates,[3] however, concluded that only virulent strains multiply well in host cells *in vitro,* while attenuated and avirulent strains show correspondingly less ability to grow. They, therefore, suggest that virulence may be a function of capacity to survive and multiply in an intracellular environment. They also observed a delay, or lag, in adaptation of the bacilli to the intracellular environment that was increasingly marked in proportion to the degree of attenuation. They used rabbit monocytes.

To explain the difference from Suter's findings they assert that certain aspects of his technique are open to criticism. In Suter's comment he says that he has made no direct comparative experiments with rabbit and guinea-pig macrophages, so he does not know if the reason for the difference may lie in that factor, but he upholds the validity of his observa-

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[1] SUTER, E. Some aspects of intracellular parasitism of pathogenic microorganisms a review. *Internat. J. Leprosy* **22** (1954) 1-11.

[2] SUTER, E. The multiplication of tubercle bacilli within normal phagocytes in tissue culture. *J. Exper. Med.* **96** (1952) 137-150.

[3] MACKANESS, G. B., SMITH, N. and WELLS, A. Q. The growth of intracellular tubercle bacilli in relation to their virulence. *American Rev. Tuberc.* **69** (1954) 479-494.

tions and conclusions. Whatever the case with *M. tuberculosis,* in leprosy we see bacilli that by all ordinary criteria are of very low pathogenicity multiply in the host cells after usually prolonged periods of latency—and often in tremendous numbers—provided they find conditions that are favorable. Precisely what those conditions are is what we would like to know.

A second point concerns the ability of monocytes from "immune" animals, either vaccinated or infected, to inhibit the multiplication of intracellular bacilli. This is by far the more important from our point of view, because it is the factor which would logically explain the essential differences between certain kinds of leprosy lesions. Suter[5] found in his monocyte cultures that the cells from immune guinea-pigs inhibited multiplication of ingested tubercle bacilli under conditions that permitted their growth in normal cells. Mackaness,[6] however, failed to find any difference in this respect between normal and "immune" rabbit monocytes.[7]

One thing that he did observe is that the immune cells were evidently sensitive to the presence of the bacilli, because they always died sooner than did the normal cells. This, it may be noted, is what might be expected by past experience of several workers, that cells from immune (tuberculin-positive) animals grown out in tissue culture are damaged by tuberculin, whereas those from normal animals are indifferent to it.

In discussing the lack of inhibition by immune cells in his experiments, Mackaness refers to contrary findings of Lurie[8] in experiments of a different type done some time ago, and of Suter[5] whose experiments were much like his own except that guinea-pig cells were used. He admits that there may be inconsistencies in that the rabbit develops only a mild degree of tuberculin sensitivity as compared with the guinea-pigs, but he nevertheless holds that the evidence for "antiblastic immunity" of the monocytes of vaccinated or tuberculous animals is inconclusive.

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[5] SUTER, E. Multiplication of tubercle bacilli within mononuclear phagocytes in tissue cultures derived from normal animals and animals vaccinated with BCG. *J. Exper. Med.* **97** (1953) 235-245.

[6] MACKANESS, G. B. The growth of tubercle bacilli in monocytes from normal and vaccinated rabbits. *American Rev. Tuberc.* **69** (1954) 495-504.

[7] On the other hand, Mackaness has observed a difference in this respect between the phagocytes of normal animals (both rabbits and guinea-pigs) and those of animals treated in a very different way, namely, by injections of a preparation of polyoxyethylene ethers (surface-acting agents) called Triton. This material in cultures of normal cells does not inhibit the multiplication of intracellular tubercle bacilli, but cells from treated animals show an "artificial cellular immunity" and bacilli in them grew very slowly or not at all. It is concluded that the monocyte itself becomes modified in some way while in the body, although it does not do so *in vitro.* Thus, in Mackaness' experience, Triton has done what specific immunization of the animal seemed not to have done.

[8] LURIE, M. B. Studies on the mechanism of immunity in tuberculosis. The fate of tubercle bacilli ingested by mononuclear phagocytes derived from normal and immunized animals. *J. Exper. Med.* **75** (1942) 247-268.

In his comment on this matter, Suter[4] points out that there are several factors which might possibly explain the differences, one of them being the degree of hypersensitivity of the animals from which the macrophages are taken. (Here would seem to be involved the question of the distinction between allergic hypersensitization, a condition which would be expected to make the cells abnormally susceptible to damage by the ingested bacilli within them, and nonallergic immunity, which might cause inhibition of multiplication without the same damage.) Anyhow, Suter points out, he and Mackaness are in complete agreement on one thing, i.e., the properties of streptomycin and isoniazid with respect to inhibition of intracellular bacilli.[9, 10]

The comment of Lurie[11] deals with certain features of Mackaness' criticism of his work that are of interest from our point of view. One point concerns the difference between "allergy" and "immunity." He points out that the existence of the former, which can be produced by a mixture of the wax and proteins of the tubercle bacillus (Raffel), does not inhibit the multiplication of the bacilli. Another point is the significance of the development of epithelioid cells. He holds that epithelioid-cell formation is a direct consequence of the destruction of tubercle bacilli by "immune" cells, that "epithelioid cell transformation of mononuclears is always associated with the intracellular destruction of tubercle bacilli."

Mackaness' disagreement with others regarding the inhibitory power of "immune" monocytes at least gives emphasis to the difficulties of this line of investigation. It has been of service in bringing to mind work done by Lurie in this field more than a decade ago, work that should not be overlooked.

Abstracts of most of the communications referred to in this note will be found in the Current Literature section of this issue. —H. W. W.

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[4] SUTER, E. Growth of tubercle bacilli in monocytes from normal and vaccinated rabbits. *American Rev. Tuberc.* **69** (1954) 1060-1062 (correspondence).

[9] MACKANESS, G. B. and SMITH, N. The bactericidal action of isoniazid, streptomycin and terramycin on extracellular and intracellular tubercle bacilli. *American Rev. Tuberc.* **67** (1953) 322-340.

[10] SUTER, E. Multiplication of tubercle bacilli with phagocytes cultivated *in vitro*, and effect of streptomycin and isonicotinic acid hydrazide. *American Rev. Tuberc.* **65** (1952) 775-776.

[11] LURIE, M. B. Growth of tubercle bacilli in monocytes from normal and vaccinated rabbits. *American Rev. Tuberc.* **69** (1954) 1059-1060 (correspondence).