THE IMPLICATIONS OF SUTER'S REVIEW OF INTRACELLULAR PARASITISM WITH RESPECT TO THE PROBLEM OF LEPROSY

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Many features in Dr. Suter's review of intracellular parasitism (7) deserve thoughtful consideration by leprologists. His careful analysis of factors which influence the intracellular fate of bacterial agents elucidates definite analogies between cultivable microorganisms and the noncultivated bacilli of human and murine leprosy. Furthermore, the picture obtained by experimental modification of cellular systems containing cultivable mycobacteria provides a new and interesting basis for examination of the more specialized problems and properties of these two types of bacilli. It is hoped that discussion of these properties in the light of Suter's illuminating review may assist in developing better understanding of the pathogenesis and chemotherapy of leprosy.

First of all, we may be grateful to Dr. Suter for having summarized the evidence that prolonged, detailed investigation in animals infected with brucellae or tubercle bacilli is an unsatisfactory way to obtain presumptive evidence whether a chemotherapeutic agent possesses significant intracellular action. He has made it perfectly plain that chemotherapeutic effects observed in such diseases may have no direct meaning with respect to the intracellular bacteria. By confining tubercle bacilli to an intracellular mode of existence, the greater efficacy of isoniazid and the poor action of streptomycin in the absence of isoniazid have been delineated as precisely as by results obtained in the treatment of mouse leprosy (1, 2, 6). The Suter system appears, therefore, to afford a second, more rapid and more analytical means of obtaining this type of information. Since *in vitro* systems are amenable to greater physiological modification in the mouse, I shall try to indicate below why proper exploitation of the Suter system may provide an eventual working model more pertinent to human leprosy than the one now provided by murine leprosy.

The evidence that intracellular environment protects brucellae and mycobacteria from certain drugs and also from antibody or other serum components is consistent with the record and with the conclusions which Clark Gray and I have reached after intensive study of the metabolic behavior and infectiousness of *M. leprae murium*. This organism as obtained from homogenates of infected tissues exhibits two outstanding characteristics: (*a*) an inability to derive benefit from substrates or

nutrients utilized by other bacteria or by tissue cells, and (*b*) an extreme susceptibility to naturally inhibitory components in serum and body fluids. As a consequence of these factors the metabolism, infectiousness and viability of this organism are impaired promptly and severely by exposure to extracellular environment *in vivo* and by exposure to the serum media ordinarily used for the cultivation of tissue cells *in vitro*. For this reason, Suter's demonstration that mild degrees of extracellular inhibition suffice to convert a cultivable mycobacterium into an intracellular parasite is an observation of prime importance to our clearer understanding of the pathogenesis of leprosy. In view of the orderly trend toward metabolic limitation and associated inhibitions among the pathogenic and parasitic mycobacteria, there seems little doubt that systems in which the tubercle bacilli would be subjected to more severe inhibition [1] should permit studies and conclusions comparable to those which may be obtained when the two types of leprosy bacilli can be propagated in cell cultures.

More detailed consideration of the properties of the noncultivated leprosy bacillus may assist in understanding the reasons why chemotherapeutic models based on mouse leprosy or inadequately inhibited tubercle bacilli in cell cultures may not provide results similar to those obtained in the treatment of human leprosy.

Dr. Clark Gray, in this laboratory, has made a careful analysis of the metabolic properties within the mycobacterial spectrum represented by saprophytes, "atypical pathogens," tubercle bacilli, Johne's bacillus and *M. leprae murium*. These investigations show that the trend toward slower growth rates, increased fastidiousness of growth requirements, and noncultivability is explained by step-wise limitations in oxidative capacity. We do not understand why or how this trend is induced by, or is advantageous to, prolonged residence in tissues. Nevertheless, my own investigation of the growth and metabolism of the same mycobacterial groups in serum and body fluids has revealed that these enzymatic limitations are expressed in part by an increasing susceptibility to inhibition in serum and body fluids. Thus, in our present views, a notable combination of metabolic limitations and of inhibitions by extracellular fluids are the main factors which force the fastidious and noncultivated species toward seclusion in intracellular environment. Two facts con-

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[1] Streptomycin was the ideal choice for Suter's purpose, but it is not adequate for the system suggested. Even concentrations greater than those employed by Suter and Mackaness, this drug does not depress the basal metabolism or viability of washed mycobacterial suspension ([5]). It renders noninfectious only those bacilli which undertake to grow in the extracellular fluids. The inhibitors in serum, on the contrary, cause much more fundamental damage to *M. leprae murium*, since they disrupt the basal metabolism on which persistence of viability depends. It is this more severe inhibition by natural body components which now prevents satisfactory propagation of *M. leprae murium* in cellular systems *in vitro* and which may make cultivation of *M. leprae* even more difficult.

cerning *M. leprae* are widely accepted and consistent with this view. These are: (*a*) the apparently very slow growth rate in tissues of the most susceptible members of the only host species, and (*b*) the failure to transmit leprosy experimentally even within the natural host. It may be suggested, therefore, that this organism differs from *M. leprae murium* in that it is metabolically less active and more readily inhibited.

If this view represents a reasonable interpretation of available facts, it seems to me that *M. leprae,* even in intracellular environment, may operate at much lower metabolic levels than intracellular tubercle bacilli or *M. leprae murium.* In that event, attempts to solve the chemotherapy of leprosy by continued search for drugs having dramatic effects may prove to be disappointing. Discussion of the mode of action of several drugs and of the serum inhibitors is to appear shortly in articles to be published in this periodical. It is necessary at the moment only to point out that low metabolic response in a microorganism imposes severe limitations on the effectiveness of antimetabolic drugs. In a search for useful alternatives capable of more decisive action against *M. leprae* we must return to the immunological studies of Suter.

His experiments show that resistance to multiplication of tubercle bacilli is a property acquired in immunized animals by the cells alone and is not dependent on serum factors. The question has been raised whether similar findings may be anticipated when such experiments can be conducted with leprosy bacilli. There is also a question whether marked differences might not be observed in comparisons between cells from tuberculoid and from lepromatous patients. The fact that *M. leprae* fails to propagate in cell cultures of fibrocytes or of blood macrophages does not permit an answer based on mycobacterial growth. Nevertheless, observations dependent on physiological processes which cause intracellular destruction of leprosy bacilli are believed to confirm and extend the findings that have been made with tubercle bacilli.

The way in which actively growing fibrocytes from tuberculoid skin lesions cause rapid reduction of *M. leprae* to acid-fast debris has been described (3). The most striking observations were made upon adding *M. leprae* to cell cultures from such lesions and which had been grown *in vitro* until free of mycobacteria (three or four months, involving many cell generations). Cells acquiring moderate numbers of bacilli were damaged; many retracted their processes until they appeared epithelioid; these exhibited "rosy" cytoplasm after staining. Even within cells acquiring smaller numbers of bacilli and retaining their spindle forms, the bacilli were segregated in vacuoles and reduced to acid-fast debris in less than 18 days. On the other hand, fibrocytes from lepromatous lesions grew normally when containing much higher numbers of bacilli, and were unable to bring about their prompt destruction (4). Thus, the behavior of cells from the two kinds of cases, even after prolonged culti-

vation *in vitro,* reflects certain of the well-known differences between the two polar types of leprosy.

Since cell cultures of blood macrophages were later found to be more destructive to leprosy bacilli than fibrocytes, it was anticipated that by study of macrophages similar or more striking observations should distinguish the two polar types of leprosy. These experiments were not completed, due to unexpected disruption of work at Culion in 1945. If these findings and anticipations are added to the observations of Suter, it appears that enhanced ability to inhibit growth and also to disintegrate mycobacteria might be shown to be associated properties of immunized cells.

Thus, if it be true that we deal with an infectious agent which already possesses an exceedingly low metabolic activity, and which is restricted largely to intracellular environment, we must be grateful to Dr. Suter for having again drawn our attention to cellular mechanisms which inhibit the intracellular growth of mycobacteria and to the existence of physiological states which encompass their destruction. It is to this type of action that the mycobacteria are ultimately vulnerable. Appropriate modification of cell response would seem to deserve much more emphasis than is now placed on this basic principle.

RESÚMEN

El autor discute las implicaciones y posibles aplicaciones del trabajo de Suter (q.v.) y hace incapié en la importancia de haber traído de nuevo a discusión las ideas y los experimentos que ponen en manifiesto la interrelación entre los microorganismos intracelulares y las celulas fagocíticas en el control de infecciones tales como la lepra.

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