THE ORIGIN OF THE CAPSULES ON *MYCOBACTERIUM LEPRAE* AND OTHER TISSUE-GROWN MYCOBACTERIA

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Babes ([1]), Unna ([8]), and other workers have questioned whether the global matrix of the clumps and globi of *Mycobacterium leprae* is produced by the bacillus or in part by interactions between the bacillus and the host. Recent studies by electron microscopy of ultra-thin sections have focused attention on the electron-transparent zones, but have not elucidated their origin or nature. Since on mycobacteria grown in tissues there may be an admixture of capsular and tissue components or a definable zone of interaction, a few chemical and physical data may help to clarify this question.

Cytological observations.—Cytologic evidence from ultra-thin sections is consistent with the view that capsules and matrices are synthesized solely by the mycobacteria, and that the zones of interaction with tissue are at the outer surface of these structures. The disappearance of such materials from *M. leprae* during sulfone therapy can best be explained on the basis that capsules are not to be expected on senescent bacterial populations ([6]), and that chemotherapeutic agents modify synthetic processes in bacteria rather than in mammalian tissues. The zones around *M. leprae* and *M. leprae murium* are not seen around unrelated infectious agents. The sharing of matrices by *M. leprae* causes clumping which is analogous to that tubercle bacilli grown *in vitro*. Futhermore, it will be noted that clumps and globi of *M. leprae* are imbedded in homogeneous masses of electron-transparent material, and that the outer surface of the globus seems to be restrained by a thin membrane ([2, 3]).

Chemical behavior.—Frank differences in the chemical and physical properties of tissue derivatives and mycobacterial matrices or surfaces afford further evidence that the electron-transparent and clumping component is purely of mycobacterial origin.

Chloroform, for example, coagulates tissue components, and is the major solvent for the waxes of tubercle bacilli. Brief shaking with only 5-10 per cent chloroform in aqueous systems is the method par excellence for declumping and dispersing *M. leprae* into suspensions comprised of single bacilli. This prompt penetration of large globi

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leaves little doubt that the classical chloroform soluble lipids of mycobacteria are the major bonding substances in the electron-transparent material. The declumping phenomenon is a characteristic of all cultured mycobacteria and of tubercle bacilli in exudates ([3]).

Digestion with 0.5 per cent bile and 1 per cent pancreatin at 37°C is a most effective means of digesting and dispersing tissue components other than collagen. If the components in question were admixtures of tissue and bacterial substances, they should be altered by this reagent. However, such digestions fail to declump *M. leprae*, tubercle bacilli, or *M. phlei*. They do not alter the dye-impermeability of *M. leprae murium*. The capsules remaining after this treatment are demonstrable by light microscopy.

Iso-electric behavior.—Evidence that tissue components are adsorbed to the capsular surfaces of tissue-grown mycobacteria and can be removed without modifying the properties expected in mycobacteria is as follows. Distinction between tissue-coated surfaces and mycobacterial envelopes is possible because of the different iso-electric points of tissue homogenates (pH 4.6) and all normal mycobacterial species studied (pH 1.5-2.0). After *M. leprae murium* has been washed in aqueous media until apparently free of tissue ([5]) and devoid of demonstrable tissue enzymes ([4]), the suspensions have always flocculated at pH 4.6 and not at pH 1.5. This indicates that such bacilli are coated with tissue components. However, after 5 minutes at 37°C in 1 per cent pancreatin at pH 7.8, these suspensions are stable at pH 4.6 and flocculate rapidly at pH 1.5. After such treatment these organisms transfer hydrogen to external acceptors at the usual very slow, persistent rates. They are infectious for animals. They possess the usual tolerance to potassium hydroxide, are resistant to permeation by safranin and tetrazoles, and stain but slowly with crystal violet.

SUMMARY

Frank differences in the chemical and physical properties of tissue derivatives and the electron-transparent materials surrounding tissue-grown mycobacteria, indicate that these structures are synthesized solely by the mycobacteria, and that tissue components occur only on their outermost surfaces.

RESUMEN

Las manifiestas diferencias en las propiedades químicas y físicas de los derivados histológicos y las sustancias electrono-transparentes que rodean las micobacterias cultivadas en tejido, indican que dichas estructuras son sintetizadas exclusivamente por las micobacterias, y que no se presentan componentes histológicos más que en sus caras más externas.

RESUMÉ

On constate des différences nettes dans les propriétés chimiques et physiques des

dérivés tissulaires et du matériel transparent au microscope électronique qui entoure les mycobactéries obtenues à partir de tissu. Les différences montrent que ces structures sont synthétisées uniquement par les mycobactéries, et que les constituants tissulaires n'existent seulement qu'à leur périphérie la plus externe.

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