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EDITORIALS

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SENSITIZATION BY CELL WALLS OF MYCOBACTERIA

To anyone concerned with the immunology of mycobacterial diseases, there is decided interest in a recent report by Larson and associates[1] on work with isolated cell walls and protoplasm of mycobacteria. Tubercle bacilli and *M. butyricum* were used in their work, the fractions were separated by mechanical means, and the electron microscope was used to check the suitability of the fractions for use.

The principal findings are, in short, that on intradermal injection in rabbits the cell walls cause delayed skin lesions and sensitization, whereas the protoplasm does not cause either. Sensitized animals react quickly to both protoplasm and cell walls.

1. In *normal* animals, (*a*), the protoplasm on injection causes transient erythematous, edematous areas that are gone within 24 hours, with no after-effect.

(*b*) With the cell walls, on the other hand, after 4-5 days there are firm, raised, red lesions, 5-25 mm. in diameter, which persist for 4-5 weeks and then fade progressively. Sensitization is established before this occurs.

2. In *sensitized* animals there is reactivity to both protoplasm and cell walls. (*a*) Testing with protoplasm causes in 24-48 hours large erythematous and edematous lesions, which are evanescent and gone in another 48 hours.

(*b*) Testing with the cell walls, injection of small doses (10γ or less) produces in 24-48 hours reaction lesions (accelerated) which are raised, hard, deep red or purple in color, and which persist for 4-5 weeks. After larger doses (20γ or more), the lesions have necrotic centers with exudate, and they heal with scars.

Other features of interest relate to dosage of the antigens, and to specificity of the reactions. About the histology of the late lesions, which also would be of interest, nothing is said.

In talking about dosage, the authors speak only in terms of gammas (i.e., micrograms, 10^{-6} gram), indicating that the fractions are highly antigenic. To sensitize a rabbit, as little as 2.5γ of the butyricum cell walls may suffice, or 10γ of tubercle-bacillus

[1] LARSON, C. L., RIBI, E., WICHT, W. C. and LIST, R. Skin reactions produced in rabbits by cell walls of protoplasm of Mycobacterium tuberculosis and M. butyricum. American Rev. Resp. Dis. **83** (1961) 184-193 (abstract in this issue).

cell walls (although larger doses were usually used). Oddly, the saprophyte thus proved to be the more effective antigenically. Of the protoplasm, doses approaching 1,000γ failed to sensitize—although undoubtetdly the Arthus phenomenon could have been produced with that material.[2] In the animals sensitized to BCG cell walls, the 48-hour reactions could often be elicited by less than a gamma of protoplasm.

As for specificity, there seems to have been none with respect to cell walls; an animal sensitized to one cell-wall preparation responded equally to the other. There was, however, a material degree of specificity with the protoplasm. For example, in one experiment tabulated, BCG-sensitized rabbits tested with small doses reacted regularly (48-hour reactions) only to BCG protoplasm, and butyricum rabbits only to the homologous protoplasm, although the matter is not entirely simple, and relatively high doses (100γ) occasionally gave cross reactions.

The late reaction to cell walls in normal rabbits has an obvious resemblance to the late, or Mitsuda, reaction to lepromin in normal persons or dogs, although nothing is said about the peculiar persistence of the reaction—or, as said, the histopathology. One can also see an essential resemblance to the delayed granulomatous lesions caused in occasional persons by zirconium and other substances, including even tuberculin, recently studied by Hurley and Shelley.[3]

About the cell-wall reaction in sensitized animals, it is not clear from the article whether or not it amounts simply to a much-accelerated late reaction, or to a combination of reactions comparable with the early (Fernandez) and an accelerated late (Mitsuda) reactions to lepromin, as occurs in highly reactive persons or sensitized dogs. About this point we are told (personal communication) that a bimodal response was not seen; "the reactions are at their height in 24-72 hours and subsequently there is a regression in their activity." This is in keeping with the fact that the time required to attain the full late effect of normals (4-5 days, compared with about 21 days for lepromin) is so short. In any event, the sensitization is clearly an allergic condition, without any relation to foreign-body effects or to any extraneous element, which have been involved by certain workers in connection with the Mitsuda reaction.

In this work the antigens were prepared from bacterial cells of fresh cultures. The cell walls of the bacilli in suspensions were ruptured by means of a pressure cell or a Mickle shaking apparatus, and the two elements were separated by centrifuging. (There is no statement about how the weights of the two fractions were determined.) Because the authors concluded that the mode of preparation used did not affect the activity of the product, it seemed to us possible that the rupture of the cells could be accomplished equally well, and perhaps

[2]The senior author (personal communication) agrees with this statement, saying that this is one of the many questions for later investigations.

[3]HURLEY, H. J. and SHELLEY, W. B. Sarcoid granulomas after intradermal tuberculin in normal human skin. Internat. J. Leprosy **29** (1961) 88-98.

more easily, by means of ultrasonic treatment, as was done recently by Rees and Valentine.[4] However, both Larson and Rees state (personal communications) that ultrasonic treatment is quite unsuitable for the purpose; the Mickle apparatus has serious limitations, and it was only when pressure systems were available[5] that really good yields of cell walls and protoplasm could be obtained.

Now it has been shown (1) that the special (delayed) reactivity of mycobacteria lies in the capsules, which (2) when separated are not acid-fast. Although the substance on which acidfastness depends (supposedly mycolic acid) goes with the protoplasm, yet it does not serve as an adjuvant to enable that fraction to cause the delayed type of hypersensitivity. The finding that tubercle bacilli rendered nonacidfast by cultivation of isoniazid medium are not capable of inducing hypersensitivity[6] would seem to indicate that the chemical changes induced by that means affect more than simple acidfastness; apparently the composition of the cell walls themselves is affected.

The authors review pertinent work in this field. Raffel[7] and Choucroun[8] established that another factor besides the protein was needed to induce delayed hypersensitivity (in the guinea-pig), and that substance was identified as a glycolipid called "Wax D"[9]—which evidently is not the mycolic acid to which acidfastness is ascribed. It has been held that if Wax D is not the essential factor, then it is a complex present in dilapidated cell walls which consist of "firmly bound lipids" ([10, 11]).

Reports by Smith and Robertsen[12] and Erickson and Smith[13] (abstracted in this issue) tell of the lack of immunogenicity of Choucroun's PMKo and of Raffel's Wax D, but retention of that character of "defatted" tubercle bacilli which retain their acidfastness. One wonders

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[4]Rees, R. J. W. and Valentine, R. C. The appearance of dead leprosy bacilli by light and electron microscopy. Internat. J. Leprosy **30** (1962) 1-9.

[5]Ribi, E., Perrine, T., List, R., Brown, W. and Goode, G. Use of pressure cell to prepare cell walls from mycobacteria. Proc. Soc. Exper. Biol. & Med. **100** (1959) 647-649.

[6]Russe, H. P. and Barclay, W. R. The effect of isoniazid on lipids of the tubercle bacillus. American Rev. Tuberc. & Pulmon. Dis. **72** (1955) 713-717.

[7]Raffel, S. The components of the tubercle bacillus responsible for the delayed type of "infectious" allergy. J. Infect. Dis. **82** (1948) 267-293.

[8]Choucroun, N. Tubercle bacillus antigens: Biological properties of two substances isolated from paraffin oil extract of dead tubercle bacilli. American Rev. Tuberc. **56** (1947) 203-226.

[9]Freund, J. and Stone, S. H. The effectiveness of tuberculoglycolipid as an adjuvant in eliciting allergic encephalomyelitis and aspermatogenesis. J. Immunol. **82** (1959) 560-567.

[10]White, R. G., Bernstock, L., Johns, R. G. S. and Lederer, E. The influence of components of *M. tuberculosis* and other mycobacteria upon antibody production to ovalbumin. Immunology **1** (1958) 54.

[11]White, R. G. and Marshall, A. H. E. The role of various chemical fractions of *M. tuberculosis* and other mycobacteria in the production of allergic encephalomyelitis. Immunology **1** (1958) 111.

[12]Smith, D. W. and Robertsen, J .A. Immunogenicity in guinea pigs of lipid fractions of Mycobacterium tuberculosis. American Rev. Resp. Dis. **85** (1962) 398-401.

[13]Erikson, R. L. and Smith, D. W. Immunogenicity of defatted mycobacteria in guinea pigs. American Rev. Resp. Dis. **85** (1962) 402-406.

what the immunologic results would be if L-bodies or protoplasts of mycobacteria could be used in comparable studies. Presumably, lacking cell walls, they would be unable to sensitize for the delayed type of reactivity.

It would be interesting to apply this line of investigation to the leprosy bacillus. Since empty capsules of *M. leprae murium* have been produced by Rees and Valentine, presumably such suspensions could be separated into the two fractions necessary for the work. The human leprosy bacillus can be separated from the leproma tissue in a considerable degree of purity by the chloroform acetone method,[14] and although as they exist in the lesions they are far different from the young mycobacteria from cultures, they could perhaps be similarly fractioned by one or another of the methods available. What would result from such work with respect to information about the Mitsuda reaction, if anything, cannot be known until it is done. The possibility that in the protein fraction might be found some degree of specificity in skin testing seems alluring, although presumably it would elicit response only in early (Fernandez) reactors to lepromin. —H. W. W.

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[14]WADE, H. W. Lepromin vs purified bacillus suspension. I. Preparation of a purified bacillus suspension. Internat. J. Leprosy **30** (1962) 19-26.