"Spherules" of Mycobacteria

A peculiarly interesting feature of the report of Merckx and associates, considered in the preceding note, is the observation of the production of peculiar "spherules" within the mycobacteria studied, both *in vivo* and *in vitro*.

These bodies, more common in the lesions of B41 mice than in those of H37Rv mice, surprisingly were not seen until several months after infection; they were particularly prominent in the liver and spleen when the lesons appeared to be regressing. The bacilli in which they appeared, it is pointed out, were in contact with the cytoplasm of the cells and not in "bags" (i.e., phagosomes). Similar bodies, which they called "sporelike structures," have been described by Brieger and Glauert in *M. avium*.[1]

Spherules may be homogenous (see the bacillus in Fig 1),[2] or finely granular, or they may contain electron-opaque granules or filaments. The spherules are apparently released by rupture of the bacillus, and some may become embedded in lysosomes (Fig. 2). It is not known what part the spherules may play in the growth and development of mycobacteria.

It may be noted, however, that the structure (granule) within the

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[1]Brieger, E. M. and Glauert, A. M. Spore-like structures in the tubercle bacillus. Nature (London) **178** (1956) 544. (Reference from Merckx *et al.*)

[2]Pictures kindly supplied by Dr. Karlson.

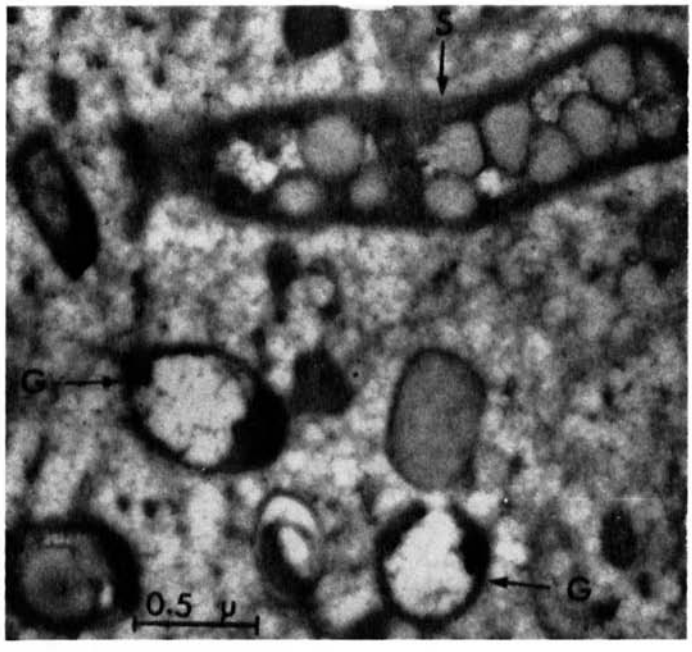

FIG. 1.—A bacterial cell filled with spherules, in the lung of a B41 mouse after 150 days of infection; separate spherules with granules are marked **G** (32,000×) (Note also—not mentioned by the authors—two spherules within what appears to be the wall of a bacillus cut transversely, in the left lower corner.)

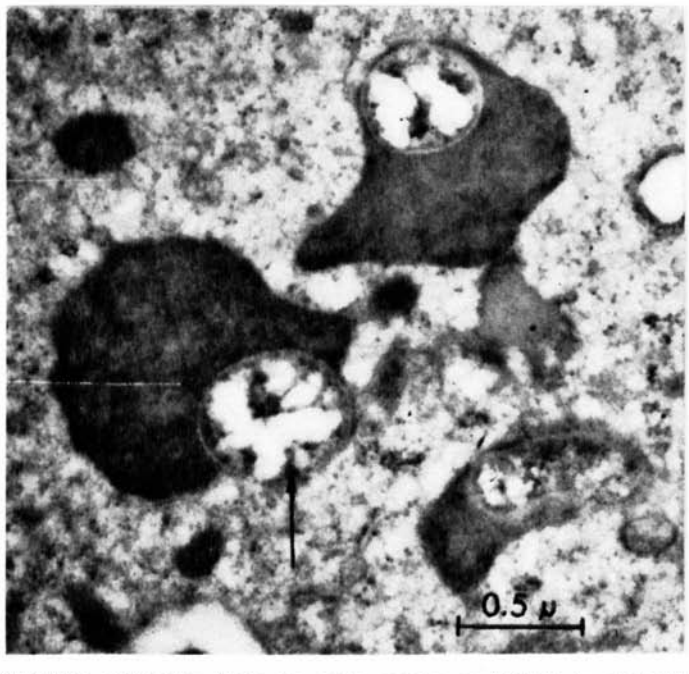

FIG. 2.—Granular spherules, from the same source as Figure 1, embedded in "opaque droplets' (lysosomes?)" (32,000 ×.) (Note the elongation of the spherule in the right lower corner.)

opaque droplet in the lower right corner of Figure 2 is elongate, as if it were a developing bacillus. That structure brings up the question of the nature of the opaque droplets, which the authors call—with some uncertainty—"lysosomes?" They point out, however, that Nishiura regards the "opaque droplets" found in leprosy lesions as being lipoid materials which accumulate around the mycobacteria.

There may also be a question whether the spherules have any resemblance to, or relationship with, the round bodies found by Imaeda in a leprosy bacillus and reproduced in an editorial in THE JOURNAL in 1963.[3] Imaeda would probably say "no," as he believed that that peculiar picture was of a complicated "intracytoplasmic membrane system" sectioned by chance in a certain way.

The authors tell of "identical structures" (i.e., spherules) produced *in vitro* by both the H37Rv bacillus and the B41 bacillus, particularly the latter when grown in the Proskauer and Beck liquid medium. They picture, at 32,000 magnification, a single B41 bacillus filled with spherules, similar to the one found *in vivo* and shown in Figure 1, except for a considerably greater variation in size of the spherules. Another picture, also taken after one day, shows bacilli filled with spherules and also free spherules, each with an electron-opaque granule. A third picture, taken after 37 days in the medium, shows only pleomorphic spherules ("granules").

If such bodies are living forms, it should probably not be difficult to determine whether or not they would, on transfer to fresh media of some kind, develop into bacillary forms. It seems passing strange that these elements were not recognized and thoroughly studied long ago.

It might be worth while to scrape pulp from the cut surface of a heavily bacillated leproma, plant it in liquid media, and with the electron microscope search for spherule production. Even negative findings might be significant, suggesting that the leprosy bacillus differs in that respect from ordinary mycobacteria.

—H. W. WADE

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[3]WADE, H. W. Undiscussed problems of the bacteriology of tuberculoid and borderline leprosy, Internat. J. Leprosy **30** (1962) 489-494.