

DISPARITY IN APPEARANCE OF TRUE HANSEN'S BACILLI AND CULTURED "LEPROSY BACILLI" WHEN STAINED FOR FAT

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By application of the writer's improved fat-staining procedure for dried preparations (Burdon, 1946) it was found that the principal varieties of the acid-fast bacilli in culture show an essentially similar picture with respect to their stainable intracellular lipid. Characteristic of the whole group is the tendency of the cells to stain *throughout* with the Sudan black B; in addition, distinct deeply colored fat droplets may be present within many of the rods. Some differences were noted, however, in the amount of fatty material usually present, and in the regularity with which it occurred, in different varieties of these organisms.

When preparations were made from parallel cultures on coagulated egg media, the 8 strains of human and the 3 strains of bovine tubercle bacilli examined usually contained a moderate number of fat droplets, but there were always to be seen numerous individual rods without stained lipid, and in some cultures none of the organisms were found to contain any material that colored with Sudan black B. Three strains of avian tubercle bacilli, however, regularly showed fatty cell inclusions in almost every mature cell. Stainable intracellular lipid was also found constantly, and more abundantly, in 2 strains of tubercle bacilli of cold-blooded animals (Friedmann's turtle bacillus and *Mycobacterium marinum*) and in 8 cultures of frankly saprophytic acid-fast organisms.¹ Finally, in all the 11 cultures of "*Mycobacterium leprae*" studied² the stain revealed an especially conspicuous content of fatty material.

The constancy and prominence with which stainable lipid occurs in these

¹ The saprophytes included cultures labeled as follows: (1) Hog skin bacillus 5138, Dr-Buckley, B.A.I. 1926, ATTC 4268, Group IIIb; (2) Zeissig's acid-fast bacillus, Cornell, 1927, Group IIIa; (3) *Mycobacterium phlei*, Lister Inst. 59, ATCC 354; (4) Butter bacillus of Rabinowitsch, Lister Inst. 524, ATCC 356, Group Ia; (5) *Mycobacterium pseudoperlsucht*, Lister Inst. 2070, Group Ia; (6) Bayne-Jones acid-fast bacillus, Group IIa (1); (7) *Mycobacterium phlei* (timothy), 223 (Pasteur Inst.); (8) *Mycobacterium phlei*, ATCC 355, E. G. Hastings' 74B. Cultures (1) through (6) were obtained from the N. Y. State Veterinary College, through the kindness of Dr. W. A. Hagan.

² Eight cultures of "leprosy bacilli," received from the National Leprosarium, Carville, La., were marked as follows: No. 111, isolated by Levy (Chrome) (Duval 114); No. 116, isolated by Neaham (64) (Ann Arbor 68a); No. 121, isolated by Clegg (Ann Arbor 0580); No. 122, isolated by Kral (Ann Arbor 0614); No. 132, isolated by Souza-Aranjo (Souza-Aranjo I); Elly strain; Barry strain; Phipps Inst. strain. Another culture obtained from N. Y. State Veterinary College was labeled Clegg I, Lister Inst. 512, 1926, Group Ib. Two additional cultures, from our stock culture collection, were marked Brinkerhoff 1, Duval 107, from Duval 105; and Brinkerhoff 2, Duval 108.

cultured "leprosy bacilli" was repeatedly confirmed, and their marked similarity in this respect to the tubercle bacilli of the "cold-blooded type," and to the frankly saprophytic acid-fast organisms, was made clear by numerous comparative tests. In the light of our finding that closely related bacteria tend to have the same content of fat-staining material (*loc. cit.*) this similarity is suggestive of a fundamental biological relationship between these organisms.

In these circumstances it seemed of special interest to apply the stain to direct films from leprous lesions, and thus to extend observations to the true Hansen's bacilli. Through the kindness of Dr. G. H. Faget, Medical Director, U. S. Marine Hospital (National Leprosarium), Carville, Louisiana, a series of unstained preparations made directly from bacteriologically positive lesions was secured. These preparations were subjected to the routine fat stain, and to various modifications of the usual technique, in order to test critically for the presence of stainable lipid in the true leprosy bacilli. The procedure finally adopted was as follows: (1) the heat-fixed films were stained with dilute carbol fuchsin only and examined microscopically to locate the leprosy bacilli (recognizable by characteristic clumps and globi); (2) several of these groups of bacilli were marked by making a ring around them on the slide with a Zeiss object-marker; (3) after removing the immersion oil by a brief dipping in xylol, Sudan black B solution was applied for 15 minutes or longer; then the smear was cleared with xylol as usual, and sometimes it was also counterstained with dilute carbol fuchsin, sometimes not; (4) the ringed areas were relocated and the organisms were carefully examined for any evidences of stained intracellular lipid; (5) the slides were again cleared of oil and subjected to the usual Ziehl-Neelsen acid-fast stain; and, finally, (6) the organisms in the ringed areas were located once again in order to confirm their identity as leprosy bacilli by their acid-fast staining. Satisfactory controlled observations of this sort were possible on a total of 15 preparations, representing 8 separate leprous patients.

The results were entirely consistent in all preparations—that is, no intracellular stainable fatty material was observed in any of the Hansen's bacilli. This finding is given somewhat added weight by our further observation that human tubercle bacilli *do* contain matter which stains with Sudan black B in direct preparations from tuberculous tissues, as well as in most cultures. The apparent total lack of stainable lipid in the true causative bacilli of leprosy is, at least, in striking contrast to the abundance of this material in the acid-fast bacilli isolated from leprous lesions and now maintained in the laboratory cultures described above. The full significance of this disparity is debatable at this time, but it would seem justifiable to count it as adding a further bit of evidence in support of the already widely held opinion that the organisms in these cultures are not identical with the true causative agent of leprosy. Their conspicuous content of stainable lipid strongly suggests a close relationship to saprophytic acid-fast bacilli.

REFERENCE

- BURDON, K. L. 1946 Fatty material in bacteria and fungi revealed by staining dried, fixed slide preparations. *J. Bact.*, **52**, 665-678.