

SOME OBSERVATIONS ON
MYCOBACTERIUM LEPRAE

Is the degree of granularity of *Mycobacterium leprae* constant in the various nodules? Does it rise and wane? Does it attain a peak in the oldest nodules? These are questions for which we have as yet no definite answer. In the light of Hoffmann¹ and Manalang's² suggestion that under treatment the rod-like bacilli become granular the above questions assume an added significance. Hansen's³ original description itself contains records of rods and granules and many who claim to have cultured these bacilli (Lowenstein,⁴ Salle,⁵ Ota and Sato⁶) describe rods, "seed rows" or "string of pearls" and granules. Hoffmann considers that Hansen's bacillus produces "in its evolutionary cycle great numbers of granular forms which are found both within the bacilli and as free lying bodies". In the case of the tubercle bacillus Kahn and Nonidez⁷ conclude that granule formation "is a type of segmentation rather than direct fission in which the rate of segmentation surpasses the ability of the elements to elongate". Marchoux⁸ states that like Hansen's bacillus "the Stefansky bacillus may break up into granules". If the suggestion that the formation of granules is an essential phase in the life-cycle is accepted, then, how are we to distinguish these from

those formed by degeneration or disintegration as a result of treatment? It will be seen, therefore, how necessary it is to have an idea of the picture presented by young and old nodules in one and the same patient.

Very recently through the kindness of Dr. A. Shama Rao, Leprosy Officer to the Government of Hyderabad, I obtained smears and biopsy specimens from a lepromatous case which presented some peculiar features which are recorded below. Dr. Shama Rao's diagnosis is as follows:—"A. K., aged 30. Lepromatous case under treatment for the last ten years. He was an L_1 case but has now become L_2 . He has nodules on the ears some of which are small and others big. A few are scattered on the body also. The face is infiltrated. There is no deformity of hands and feet and neural and acroteric symptoms are absent." On Sunday, 18-6-1944, a large nodule from the lobe of the right ear was removed and after making a few smears was fixed in Regaud's fluid. Again on Wednesday, 21-6-1944, a small nodule from the left ear was clipped and a few smears made. All the smears were stained in Ziehl-Neelsen and counterstained with Löffler's alkaline methylene blue.

In the smears made on Sunday from the large nodule, the following picture was observed. The bacilli in the globi are irregularly scattered, and those at the periphery have often a concentric arrangement. Though in most of the globi no stainable content other than the bacilli occur, yet in a few large ones where the clumped masses of bacilli occupy half or three-fourths the area of the globi only, the bacteria-free portion is stained by methylene blue and present a granular appearance. The walls of the globi are well defined and nuclei may be seen sticking to the walls. But whether these nuclei belong to the globi could not be made out from the smears. The smallest globi are less than 7μ in size and are generally spherical. In the smear many empty spaces comparable to globi but devoid of bacilli could be seen. Since the outer limiting membrane of these clear spaces appears to be incomplete it seems as if these are globi which have got ruptured during the smearing process.

The bacilli in these globi are short rods, the longest of which is 5μ long. But these long ones are rare as also granular forms. Very few—one in each field—of the long rods showed any beaded appearance. In one or two in each field there was just the suggestion of development of a bead at one end. In some regions the alignment of the short rods suggest as if they have separated from a beaded chain.

Typical "cigar packs" without any limiting membrane could be observed in various regions of the smear. These are always composed of long bacilli. From comparison with larger bundles all these long packs of bacilli could be arranged in a linear series. Single long bacilli lying free have lightly stained halos which do not completely envelope them. When two long rod-like bacilli lie side by side,

they appear enclosed by the halo. In some globi which present the typical 'cigar pack' arrangement, one can see gradations from long rods $7-8\mu$ long with a very faint suggestion of beading, to the smallest granules. Rarely single rods taper to the ends, and sometimes a beaded rod alone may be seen with a clear vacuole.

In the smallest globi measuring $5-6\mu$ short rods have a peculiar arrangement. They form a regular row at the periphery, with one or two lying in the middle. Denney¹ describes longitudinal splitting and branching but no such forms were found in the smears.

Except in the larger globi, where the center of the globus stains deeper with methylene blue suggesting a definite membrane, the smaller forms there is no such uniform differential staining. Often bacilli projecting into the blue stained cytoplasmic area suggest that the membrane is a late formation. Only careful study of serial sections would clarify this question. Irrespective of the size of the globi, the bacilli seen in them differ in shape. Some are packed with small rods and others with longer ones. The majority of course belong to the former category and only one in fifty to the latter. In every hundred of these globi show arrangement of short rods suggestive of the fact that they may have belonged to chains. In others, the arrangement is very irregular.

In the smaller nodule from which the smear was made on Wednesday the appearance of the bacilli is entirely different. About 90 per cent. in any field are long beaded ones, the other 10 per cent. being composed of short rods without beads, short rods and granules. Bacilli possessing from 2 to 8 beads could be observed in any field and even the alignment of the short rods and granules suggest as if they have just separated from chains. This impression is accentuated by the fact that in some fields with variable number of beads and free granules or small rods aligned in the same longitudinal plane could be seen in any field. In other fields of the globi are packed with these "seed rods" which if carelessly stained give the impression of a clump of grains.

It would be seen from the above description that though the degree of granularity may be said to be constant for a single nodule it is not constant for all nodules in the same patient and neither does it reach a peak in the old nodules. One is led to agree with Cowdell² that "though a single biopsy specimen may exhibit marked granularity, this may or may not be a favourable sign". When we consider that the patient has been under treatment for ten years and when different nodules show different pictures, the suggestion that under treatment the rod-like bacilli become segmented and granular and that such an appearance indicates a favourable prognosis appears to be of questionable validity.

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