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EDITORIALS

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MORPHOLOGY OF DEAD AND LIVING LEPROSY BACILLI

For some time, certain British workers have been endeavoring, by employing electron microscopy, to ascertain if the microscopic appearance of the leprosy bacilli from lesions can be correlated with their viability; in other words, if dead and degenerated bacilli can be distinguished morphologically from living ones.^{1, 2, 3} The most recent contribution, by Rees and Valentine, appears in this issue.

The murine leprosy bacillus has been used mostly, but also the saprophytic *M. phlei* to some extent, for comparison with the human leprosy bacillus. The saprophytic mycobacteria has the advantage of easy cultivability, and morphologic changes can be correlated with viability. The murine bacillus will produce lesions in fresh animals, and the ability to do so can be correlated with morphology.

As a source of material, progressive lesions of murine leprosy have the advantage that, in general, the bacterial population is actively multiplying, more or less as they would be in a growing culture, and therefore are composed mostly of viable forms. Such forms stain solidly with carbol-fuchsin, and have a corresponding solid, full appearance with the electron microscope until they undergo degenerative changes. In contrast, the bacillary population of the usual human lepromatous lesions is a very mixed lot, ranging from solid young ones (the "resistant"

¹ McFADZEAN, J. A. and VALENTINE, R. C. An attempt to determine the morphology of living and dead mycobacteria by electron microscopy. (Preliminary communication.) Trans. VIIIth Internat. Congr. Leprol., Tokyo, 1958; Tokyo, Tofu Kyokai, 1959, pp. 89-90.

² McFADZEAN, J. A. and VALENTINE, R. C. The value of acridine orange and of electron microscopy in determining the viability of *Mycobacterium leprae murium*. Trans. Roy. Soc. Trop. Med. & Hyg. **53** (1959) 414-422.

³ REES, R. J. W., VALENTINE, R. C. and WONG, P. C. Application of quantitative electron microscopy to the study of *Mycobacterium lepraemurium* and *M. leprae*. J. Gen. Microbiol. **22** (1960) 443-457.

kind which may survive the defatting effect of xylol when that is used for removing the wax from paraffin sections), through the various grades of "granular" forms that are to be seen, to old decrepit forms that may be difficult to make out in stained smears.

In this connection, it may be noted, there exists a matter of terminology which may be confusing, in the terms "granulation" and "beading." With the tubercle bacillus—and presumably it is equally applicable to the leprosy bacillus—"beading" refers to *swollen* parts or segments of bacilli which Yegian⁴,⁵ showed to be a mobile feature which can be shifted from part to part, abolished entirely, or reestablished at will. In the conditions of "granulation," however, the stained segments of the bacillus—which segments may be few or many—are not thicker in diameter than the rest of the bacillus and so do not cause swelling; and the granules are not manipulable as the beading condition is. When Rees and Valentine speak of "beading" of the leprosy bacilli, it would seem that they refer to this "granulation."

The authors and their colleagues have become convinced that certain appearances revealed by the electron microscope signify that the bacilli are dead, and that the structures found are only their relatively indestructible corpses. The problem of proof of lack of viability, it is believed, can be solved with the murine leprosy bacillus, comparing morphology and pathogenicity. Assuming that the same thing is true of the human leprosy bacillus, the problem of practical application of the findings in ordinary leprosy work—since electron microscopy is very much a special facility which is generally not available—lies in deciding whether the findings with light microscopy are comparable to those with electron microscopy.

As an approach to that problem, Rees and Valentine tell of a most ingenious stunt, whereby they have done what at first flush might be thought to be impossible: that is, they have examined individual bacilli by both light and electron microscopy. To accomplish it, smears of bacillus suspensions are made on prepared special seven-hole platinum screens used for electron microscopy and then stained with carbolfuchsin. Under the light microscope the bacilli in the few apertures of the screen are studied and their morphology sketched for identification. The same screen is then examined by the electron microscope, in which the morphologic appearances of the individual bacilli seen in the screen holes are compared with their appearances with the light microscope. The carbol-fuchsin staining apparently does not affect the results.

As an incidental but significant experiment, Rees and Valentine treated two varieties of mycobacteria (the murine leprosy bacillus and

⁴ YEGIAN, D. and BOISDEN, L. Factors affecting the beading of the tubercle bacillus stained by the Ziehl-Neelsen technique. *J. Bact.* **44** (1942) 667-672.

⁵ YEGIAN, D. and KURUNG, F. Morphology of the tubercle bacillus; a study of artifacts produced in smears. *American Rev. Tuberc.* **56** (1947) 36-40.

M. phlei) by sonic vibration to obtain preparations of empty cell walls. These were demonstrable by the electron microscope, but not by the light microscope. Carbol-fuchsin staining demonstrated no cell walls, only a background of extremely fine acid-fast granules, or particles. Thus was demonstrated, again, as it had been for example in the work of Yegian referred to, that acidfastness resides in the cytoplasmic element of the mycobacteria and not in the cell wall or a capsule.

From the finding that the morphology of the bacilli is similar by the two types of microscopy, it follows that by careful study of the bacilli in ordinary bacteriologic smears it should be possible to decide what proportion of them are alive and what proportion may be considered nonviable. This may prove to be of great practical importance with respect to treated cases in the antileprosy campaign where is followed the principle enunciated by the Tokyo congress⁶ that "*it is more advantageous to reduce infectiousness in many patients than to eliminate infectiousness in a few.*"

On this basis it would be justified to treat even lepromatous cases on an outpatient basis, at least once the bacilli in their lesions have undergone a sufficient degree of degeneration. Where it is required that bacteriologically-positive patients be hospitalized, it would not be necessary to wait until smears are negative before releasing them to their homes; they might be released as soon as the smears persistently show only the nonviable forms—with, of course, due precautions against relapses by means of continuous treatment. This would be in line with the practice of "medical discharges" that are granted under certain conditions by the U. S. Federal Leprosarium at Carville, La. This point of view is very different from the old one still prevalent in certain countries where not only negative smears are required, but after that stage is reached bacteriologic negativity of biopsy specimens as well! —H. W. WADE

⁶ [TOKYO CONGRESS] Technical Resolutions. Epidemiology and control. Trans. VIIIth Internat. Congr. Leprol., Tokyo, 1958; Tokyo, Tofu Kyokai, 1959, p. 480.

A NEW SENSATION¹

The traditional medical school explanation of cutaneous sensations is no longer satisfactory. While it relates touch sensation to Meissner corpuscles, heat to Ruffini end-organs, cold to Krause end-bulbs, and pressure to Vater-Pacini corpuscles, no specific evidence for this has ever existed. Now new histologic and histochemical techniques have demonstrated a different and simple spectrum of nerve endings in the

¹ Editorial, reprinted by permission, from the *Journal of the American Medical Association* 176 (1961) 944-945 (June 17).

skin, characterized by remarkable similarities to each other.² The dermal nerve network, the hair follicle nerve network, the mucocutaneous end-organ, the Meissner corpuscle, and the Vater-Pacini corpuscle are the nerve endings in the human skin which have morphologic distinctness. Almost all the nerve tissue of the dermis is found in the dermal and hair follicle nerve networks. The three more specialized "organized" endings are found only in the transitional zones between hairy and nonhairy skin, and in the distal grasping surfaces.

A basic pattern is common to the dermal nerve networks, the hair follicle nerve network, and the organized endings. A random association of nerve rami from many dorsal root ganglion cells occurs at all levels in the skin and ultimately supplies the endings. It is not possible to stimulate just one dorsal root ganglion cell in normal sensory experience. Many are fired by every stimulus. It is this pattern of nerve activity which allows spatial recognition and characterization of the sensation.

Pain is associated with the dermal nerve networks, but then all sensations may be associated with such simple patterns. In more rudimentary forms of life, the nerve network is the only ending present, and it retains the capacity to recognize touch and temperature changes, as well as disagreeable stimuli. All the other end-organs described are associated with the sensation of touch and are supplied by large myelinated fibers. Thus, all sensations are associated with one rudimentary pattern of nerve endings—the dermal nerve network; conversely, all the nerve endings are associated with one sensation—touch. This points out clearly the lack of morphologic specificity. Structure does not indicate function, but only the local opportunity in the skin for sensory nerve tissue to express its growth potential.

Histochemistry is adding to our appreciation of nerve endings, and some species may be characterized by the histochemical content of the cutaneous end-organs. In the cat and all other known felines, the mammalian end-organ and the Vater-Pacini corpuscle contain alkaline phosphatase. This gives their skin a distinctive appearance quite different from that of any other group of animals. The gibbon possesses alkaline phosphatase in its Meissner corpuscles, and is the only primate from the tree shrew through man which has this enzymatic reaction. A singularly unitarian chemical quality of nerve endings is the cholinesterase reactivity in all the organized end-organs. It is apparent that cholinesterase does not have a direct relationship to function, and its presence has not been explained. The Vater-Pacini corpuscle, which is found everywhere in the skin of the birds near the feather follicles, contains an acid mucopolysaccharide, a unique circumstance.

² WINKELMANN, R. K. *Nerve Endings in Normal and Pathologic Skin*. Publication 380, American Lecture Series. Springfield, Ill.: Charles C. Thomas, 1960.

The life history of the nerve endings has given useful information also. Since nerve networks are formed long before other cutaneous structures, their role in directing cutaneous development may be great. The more complex nerve endings develop fully only in postnatal life. In the erogenous mucocutaneous zones, there may be a developmental basis for the anal, oral, and genital eras of early life noted by psychiatrists as the end-organs in each region mature. As tissue ages, nerve concentration generally decreases, but very slowly. With marked activity of the grasping surfaces, it seems possible that the nerve endings become more complex. Physical activity may be one way in which the tactile discs described by Merkel and Ranvier appear.

The next task in sensation research appears to be a study of sensory patterns, not by punctate stimuli but by patterns of stimuli. It is also necessary to elaborate some interpersonal means of communication that will express sensory qualities better than the rudimentary terms used at present. Direct microelectrode studies on the end-organs might be of interest, but, on the basis of present studies, the findings of such work have been predicted. It seems at least as if we may be ready to proceed from anatomy to physiology of sensation.