INTERNATIONAL JOURNAL OF LEPROSY

Volume 46, Number 2
Printed in the U.S.A.

REPRINTED ARTICLE

Cytological Studies on Globi in Leprosy [1, 2, 3, 4]

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For over 50 years the nature of large globular masses of bacilli called "globi" has been in question. Despite the fact that they are among the most conspicuous objects observed in the lesions of lepromatous leprosy the controversy about them started by Unna and Hansen is still unsettled. Unna and his associates claimed that the bacilli, arranged in globi, are located mainly extracellularly, especially within lymph spaces, while Hansen, Neisser and others contended that the globi are mainly intracellular accumulations of bacilli. The literature to 1911 has been well summarized by Gurd (1). In 1934 Denney (2), at that time Director of the U.S. Marine Hospital (Leprosarium) at Carville, said that although they are "of perhaps the greatest diagnostic importance" their true nature remains undetermined. This was a year after he and Eddy (3) had presented a paper on the subject. In the discussion of this paper many ideas were expressed. Duval remarked that the view held by Unna prevailed until Gurd showed that the "globi" are not actually within the lymphatics but in large endothelial cells (the so-called 'lepra' cell) of the lesion. Wade, in discussing the question with European dermatologists interested in leprosy, said that he had to agree that "such masses may occur in lymph spaces" but he concurred with Denney and Duval that normally they are present in the cells mentioned. It appears, therefore, that in Europe the conception of Unna was held as late as 1934, while in this country the majority opinion was that the globi are essentially intracellular formations. It was in an attempt to secure more information that the study reported in this paper was undertaken.

I. MATERIAL

Data concerning the principal tissues examined are given in Table I. Colonel S. Simmons, when in Panama, obtained Case I from Dr. Kurevitsch. My associate, Dr. L. F. Heimburger, collected Cases 2-7 at the U.S. Marine Hospital (Carville) with the permission of Dr. O. E. Denney. He also contributed Cases 8-12 which he had secured at the Cheeloo Leprosarium in China. Cases 13-20 were collected by me at the Insular Leprosarium in Puerto Rico while I was working in the nearby School of Tropical Medicine under invitation from the Director, Dr. G. W. Bachman. Of these, biopsy in Cases 16-18 was done by Dr. J. Noya Benitez and in Cases 19-20 by Dr. F. Hernandez-Morales. Cases 21-29 were autopsies, the preparations from which were placed at my disposal, during a visit to the U.S. Marine Hospital, by Dr. H. E. Hasseltine, Director, and Dr. Sam H. Black, pathologist. The latter gave me blocks of tissue of special interest for detailed study in St. Louis.

Perspective was gained by examination of biopsies of 10 cases of tuberculoid leprosy in children, sent from the Virgin Islands by Dr. James Knott, because in this type of leprosy globi are not ordinarily found, and it is important to contrast the kinds of lesion in which they do and do not develop. Opportunity to study globi in water buffalo leprosy was afforded by a series of formalin-fixed tissues provided by Dr. H. W. Wade who se-

[1] Received for publication November 15, 1939.

[2] Aided by a grant from the U.S. Public Health Service.

[3] Reprinted with permission from the AMERICAN JOURNAL OF PATHOLOGY. Volume **16**, Number 2, March 1940, pp 103-136. A recently uncovered memo from a note by Dr. H. W. Wade, late editor of the IJL, recommended reprinting of this manuscript, it being the best study of globi of which he was aware.

[4] Current interest in the growth patterns of *M. leprae* in experimental animals, notably the armadillo, invites comparison. We have seldom seen this work referenced and it may not be readily available to many leprosy workers.

[5] E. V. Cowdry, B. S., Ph.D., from the Anatomical Laboratory, Washington University School of Medicine, and the Barnard Free Skin and Cancer Hospital, Saint Louis, Missouri.

[6] Deceased (see obituary, this issue, p 216).

TABLE I. *Clinical History.*

Case number	Age	Sex	Race	Type of leprosy*	Duration of lesions	Region of specimen	Kind of treatment	Origin of specimen	Autopsy or biopsy
	yrs.				*yrs.*				
1	70	M	Negro	Cutaneous C_3	10	Thigh	Chaulmoogra 10 + yrs.	Panama	Biopsy
2	42	M	Mexican		14			Carville	Autopsy F. S. 1015
3	42	M	Mexican	C_2N_1	4	Ear lobes	None	Carville	Biopsy P. A. 1049
4	30	M	White	C_2N_1	10	Forearm	Routine Chaulmoogra	Carville	Biopsy W. M. 619
5	36	M	Mexican	C_2N_2	5	Forehead	None	Carville	Biopsy F. H. 971
6	35	M	White	C_3N_1	19	Nape of neck	Routine Chaulmoogra	Carville	Biopsy F. V. 514
7	30	M	Mexican	C_3N_1	8	Face and forearm	None	Carville	Biopsy J. P. 1050
8	16	M	Chinese	C_1	2	Upper arm	None	China	Biopsy G 34981
9	19	M	Chinese	C_1N_1	5	Forearm	Irregular	China	Biopsy G 35846
10	31	M	Chinese	C_2	5	Forearm	None	China	Biopsy E 72466
11	45	M	Chinese	N_1	1	Upper arm	None	China	Biopsy G 40015
12	30	M	Chinese	N_2	3	Lower leg	None	China	Biopsy G 55760
13	44	F	Negro Indian	Cutaneous nodular	7	Forearm	Chaulmoogra oil	Puerto Rico	Biopsy C. M.
14	17	M	Puerto Rican	Cutaneous nodular	9	Forearm	Chaulmoogra + methylene blue	Puerto Rico	Biopsy R. R.
15	12	M	Negro	Cutaneous nodular	4	Ear crest and lobule	Chaulmoogra	Puerto Rico	Biopsy R. P.
16	22	M	Puerto Rican	Cutaneous nodular	9	Ear	Chaulmoogra orally, esters injected	Puerto Rico	Biopsy P. V.
17	25	M	Puerto Rican	Cutaneous nodular	18	Forearm	Chaulmoogra	Puerto Rico	Biopsy E. B. G.
18		M	Puerto Rican	Cutaneous nodular		Alae nasi		Puerto Rico	Biopsy E. U. C.
19	23	M	Puerto Rican	Cutaneous nodular	9	Forearm	Chaulmoogra orally, esters injected	Puerto Rico	Biopsy G. R.
20	35	M	Puerto Rican	Cutaneous nodular	16	Forearm	Chaulmoogra	Puerto Rico	Biopsy P. J. G.

TABLE I. *(Continued)*

Case number	Age	Sex	Race	Type of leprosy*	Duration of lesions	Region of specimen	Kind of treatment	Origin of specimen	Autopsy or biopsy
21	*yrs* 42	M	White	Mixed	*yrs.* 10	Several	Chaulmoogra 4 years	Carville	Autopsy No. 723
22	62	M	White	Mixed, cutaneous predominating	21	Several	Chaulmoogra 8 yrs. Fowler's sol. 1 yr.	Carville	Autopsy No. 708
23	64	F	White	Mixed, cutaneous predominating	25	Several	Chaulmoogra 16 yrs.	Carville	Autopsy No. 552
24	29	M	White	Mixed	12	Several	Chaulmoogra 2½ yrs.	Carville	Autopsy No. 880
25	53	M	White	Mixed, cutaneous predominating	17	Several	Chaulmoogra 6 months	Carville	Autopsy No. 1057
26	54	M	Chinese	Mixed	About 21	Several	Chaulmoogra and Fowler's sol. 8 yrs.	Carville	Autopsy No. 165
27	40	M	Negro	Mixed	7	Several	Chaulmoogra 1 yr.	Carville	Autopsy No. 931
28	42	M	White	Mixed	About 21	Several	Chaulmoogra 1 yr.	Carville	Autopsy No. 775
29	25	M	White	Mixed	About 17	Several	Chaulmoogra 3 yrs. Mercurochrome 1 mo.	Carville	Autopsy No. 339

*The diagnoses of clinical types of leprosy are listed as given by the clinicians kindly supplying materials. Some employed Leonard Wood Memorial Conference letters (see *Inter. J. Leprosy*, 1934, **2**, 329-356), while others did not. To attempt to bring all under a single method of classification would introduce errors.

cured them from Dr. L. W. M. Lobel. Dr. A. Zeissig, acting for Dr. W. A. Hagan, donated tissues from Johne's disease and Dr. Margaret Smith material from human tuberculosis.

To all of these individuals I wish to express my thanks.

II. TECHNIC

Differences in the bacilli, or in the lesions, owing to variations in technic, are not likely to occur. In an experiment repeated three times it was not possible to detect any significant difference in the bacilli depending on whether the tissues were fixed in:

Regaud's fluid
Zenker less acetic acid
Zenker + 5 per cent acetic acid
Zenker + 10 per cent acetic acid
Zenker less acetic acid + 10 per cent formalin
95 per cent alcohol + 10 per cent formalin
10 per cent formalin alone

Osmic acid mixtures (Bensley's A.O.B., Altmann's fluid and 1 per cent and 2 per cent osmic acid) gave good preservation of the bacilli but penetrated so poorly as to limit their usefulness. Saturated aqueous mercuric chloride plus 5 per cent acetic acid and saturated 80 per cent alcoholic mercuric chloride plus 5 per cent acetic acid gave good results. Simple fixation in 80 per cent alcohol, which is usually regarded as the best fixative for acid-fast bacilli, gave beautiful preparations of the bacilli at the expense of the tissues, which were poorly preserved. The freezing and drying technic (4) is recommended for leprosy bacilli but it is a slow method and is not feasible except in a laboratory equipped with a cryostat.

Slight delay in the fixation of tissues after their removal did not lead to alterations in

the bacilli. For example, a nodule was excised, part of it immediately fixed in Regaud's fluid, and the remainder fixed in the same way after it had been kept in a Petri dish with cotton moistened with physiological saline solution in the ice box for 16½ hours and after this at room temperature for 11½ hours. The bacilli in stained sections of both were compared. They appeared if anything to be better preserved and stained after the delayed fixation but this may be, as in the case of pure alcohol fixation, because the tissue components suffer and relative to them the bacilli are in excellent condition.

When, however, the surface of the tissue was allowed to dry before fixation the dehydration caused profound alterations in the globi and other structures. Pinching of the tissues with forceps, and to a less extent cutting with scissors, also produced confusing modifications and for this reason the tissues were lifted and cut with a wet razor blade.

The Ziehl-Neelsen method of staining acid-fast bacteria with fuchsin was employed except that hematoxylin was used as a counterstain in place of methylene blue because of its greater permanence. Precipitation of the fuchsin on the sections was prevented by covering them with a layer of filter paper before application and heating of the stain. The "new fuchsin" recommended by Fite ([5]) was tried but improvement over results obtained with other fuchsins was not great. Carpano's ([6]) technic for staining the bacilli dark violet in a yellow background gave beautiful preparations. For the coloration of both bacilli and fat in the same preparations the following method was used.

1. Cut frozen sections 10 to 15 μ thick.
2. Stain sections on the slide with carbol fuchsin with the aid of heat for 20 minutes.
3. Differentiate with acid alcohol until the sections are a dark pink.
4. Wash in distilled water.
5. Counterstain with Harris' hematoxylin for 10 minutes.
6. Differentiate in acid alcohol.
7. Neutralize with very dilute ammonia water. The sections should become blue.
8. Wash well in distilled water.
9. Stain for fat in a saturated solution of Sudan IV (scarlet red) in 70 per cent alcohol for from 1-3 hours.
10. Rinse in 70 per cent alcohol.
11. Rinse in distilled water.
12. Mount sections on a slide with glycerin and seal around the coverslip with a beeswax-balsam compound.

In order to make sure that no bacilli were missed, particularly non-acid-fast ones, Giemsa's stain was frequently applied to the sections. Except when undue pressure was exerted on the cover-glasses, no signs were found of the displacement of bacilli in the globi so that the method advised by Lobel ([7]) (in water buffalo leprosy) to hold them in place was not used.

III. OBSERVATIONS

The ideal way would have been to study the sequence of changes in the development of globi and to closely relate the changes observed to the evolution of the lesions. This, however, could not be done owing to the impossibility of collecting specimens in the latent period and the lack of clinical data as to the age of the particular nodules which were biopsied. Data on the duration of the disease in the several cases is not sufficient, since a single patient may show on his arms, for example, nodules of quite different age and structure.

Instead, the approach has been to consider: the distribution of bacilli, whether extracellular or intracellular; the formation of small and of giant globi; the significance of globus formation; and the intracellular behavior of other acid-fast bacteria as compared with that of *Mycobacterium leprae.*

I. Distribution of Bacilli

Sufficient specimens from deep lying tissues, collected at autopsy, were not studied to permit a detailed statement of the distribution of bacilli throughout the whole body. The following remarks relate only to the skin as observed in a large series of biopsies.

Extracellular bacilli must obviously exist in any growing lesion because additional cells could not become involved except by the entry of bacilli which before entry were extracellular. Usually they are difficult to find, but when pressure is exerted on the cover-glass bacilli, which were intracellular *in vivo,* may leave the cells in small numbers. Similarly extracellular bacilli, near the edges of sections, are to be interpreted with caution for a certain amount of injury is unavoidable in excising the specimens.

Nerve twigs were observed in some of the sections and a few extracellular bacilli were noted between the fibers, but no attempt was made to gather evidence on Muir's (8) contention that the reservoir of organisms is neural.

Small lymphatic vessels, at the periphery of some of the nodules, exhibited in rare cases sparsely scattered extracellular bacilli. Large lymphatic vessels were not examined in autopsy specimens. Reference will be made later to the sinuses of lymph nodes.

Within the lumens of blood vessels, especially capillaries and venules, extracellular bacilli were noted but they were never numerous and often extremely difficult to find.

Extracellular bacilli were regularly seen in the liquefying centers of lesions where there is much cellular disintegration coupled with marked invasion by neutrophils. Even in this situation there were no traces of the extracellular development of globi.

Undoubtedly the bacilli are overwhelmingly intracellular. The cells that possess them may be listed in three categories. The first includes those in which the bacilli most commonly occur. Chief among these are the monocytes, sometimes rather loosely referred to as large mononuclear leukocytes, endothelial leukocytes and even as macrophages. Intravascular monocytes laden with bacilli can be studied as individuals and are easily identified.

It is the majority of the extravascular cells, containing simple nuclei, reminiscent of those of typical monocytes, and considerable numbers of bacilli that are most difficult to classify. This is not surprising because there is little uniformity of opinion among cytologists as to the kinds and sources of the cells found in any area of inflammation even though it has been experimentally produced by a known excitant and the stages in its development have been followed from the beginning. In human leprosy we are at a great disadvantage since the lesions, when examined, are of long standing and many of the cells are more distorted by the enormous numbers of bacilli they contain than are similar cells in any other human disease. It would not be so confusing if the swelling were equal, but cells sometimes multinucleated and engorged almost beyond recognition exist side by side with others containing comparatively few bacilli and they are usually all closely pressed together.

Whether the cells with bacilli are monocytes which have invaded the tissue fluids from the blood stream, or monocytes which have developed from extravascular lymphocytes, or histiocytes which have taken up bacilli, or whether they are derived from all three sources may be impossible to determine. The fact that leprologists with one voice remark on the infrequency of cell division in nodules that are enlarging is weak evidence that the increase in cells is occasioned more by cellular immigration than by division of resident tissue cells.

To call the cells engorged with bacilli *epithelioid cells* is misleading. They are only like stratified epithelium in being close together and they are obviously not epithelial. To refer to them as *macrophages,* or "big eaters," is more justified, but they do not as a rule digest the bacilli which they contain. They range all the way from cells of small size with a single nucleus to giant cells with many nuclei.

In the so-called "foam," Virchow or *lepra cells,* which are often multinucleated, there are many bacilli but the bacilli are in some cases degenerating and there is a good deal of fatty lipoid material. These cells appear to be formed from macrophages but it is not certain that some of them are not derivatives of fibroblasts which are frequently heavily charged with bacilli.

Bacilli commonly occur also in the *endothelial cells* of blood vessels. These differ from the "special endothelial cells" of the liver, spleen and certain other organs in that they are not parts of the reticuloendothelial system.

Neutrophilic leukocytes, which invade the centers of nodules becoming necrotic, almost invariably exhibit bacilli. When, however, they are in the blood stream or in extravascular situations where there is but little necrosis bacilli are not so regularly seen in their cytoplasm.

The second group of cells comprises those that only occasionally contain bacilli. *Epithelial cells* of the epidermis, hair follicles and sweat glands come under this category. Also, bacilli are sometimes observed in small numbers in the peripheral cytoplasm of fat cells. There is some doubt as to whether the endothelial cells of lymphatic vessels and the mesenchymatous cells of the perineurium, epineurium, and endoneurium, in which

bacilli are seen, should be listed here or in the first group for which they might qualify had the search been more intensive.

The third group of cells, which are not known to harbor the bacilli in their cytoplasm, requires particular care, for the possibility must be borne in mind that in unusual lesions, complicated in various unexpected ways, they may possess them. I have not encountered any leprosy bacilli in typical *lymphocytes,* either in the blood or extravascularly in the tissue fluid. Others have emphasized their absence in lymphocytes. This is interesting because some leading cytologists believe that monocytes are developed from lymphocytes and monocytes harbor the bacilli more regularly and conspicuously than any other kind of cells.

No bacilli were observed in *plasma cells* of the Marschalko type although such cells are often abundant in the lesions. However plasma cells, as described by Unna, do contain bacilli for Unna included cells which now go under the name of monocytes in his category of plasma cells.

Russell body cells occur only rarely in cutaneous leprous nodules and when they were seen no bacilli were found within them.

Basophilic leukocytes were not observed within any of the blood vessels cut in sections. It is improbable that they carry bacilli. *Tissue basophils* were fairly numerous in the less dense lesions. No definite bacilli were detected in their cytoplasm but a very small proportion of them contained one, seldom more, fuchsin-stained, acid-resistant droplets which may possibly have been of bacillary origin.

Neither were bacilli noted in blood or tissue *eosinophils* but I am, of course, not ready to state that they never occur in these cells. The same holds for the cells of the *sheath of Schwann.*

This comparison of the distribution of extracellular and intracellular leprosy bacilli permits some rather evident conclusions. It indicates that bacilli, which are for some reason outside of cells, do not ordinarily remain long in this situation or multiply rapidly in it, but promptly become intracellular, because otherwise we would expect them to be much more numerous. Their occurrence in some kinds of cells and their absence, or very occasional presence in others, is not simply a question of entry into cells with which they happen to come in contact, for lymphocytes and plasma cells, devoid of bacilli, occur side by side with monocytes which possess them. Nor is the evidence sufficient to agree with White ([9]) that the association between bacilli and monocytes depends on the possession in common of some chemical factor "through which they contend for the same food supply." To single out thus the monocytes and ignore the other cells, within which the bacilli are found, is unjustifiable. Moreover, reasons have been given why this explanation is probably not valid ([10, 11]).

To speak of the bacilli as invading the cells which they come to inhabit is not justified because to do so implies action on their part in overcoming some sort of cellular resistance. Leprologists are agreed that the bacilli, seen in mounts of living tissue, are not motile. It is known that killed rat leprosy bacilli injected into rats become intracellular and cause lesions which resemble those brought about by living bacilli, except that they are of course limited and do not progress since there can be no multiplication of the dead bacilli (Lowe ([12])). It is therefore improbable that living human leprosy bacilli are incorporated in the cells any more actively than dead bacilli by virtue of their vitality.

As for the cells, their part may not be exactly what we would call "active." The epidermal cells are sessile and do not move about and seek out the bacilli. Endothelial cells are naturally more in the path of the organisms. Whether motile leukocytes actually make their way to them remains to be determined. The bacilli sink into the cytoplasm of some kinds of cells and not into others, and this is conditioned by the properties of the bacterial and cellular membranes.

The number of bacilli in any particular cell is not necessarily a measure of the number taken in. It is a function, not only of the intake but also of the rate of destruction of the bacilli by the cells, on the one hand, and of multiplication of bacilli in them on the other. Neither should the factor of time be ignored. The same rate of change over a long period will produce a greater result than over a shorter one. The life spans of the different sorts of bacilli-laden cells have not been determined, but they are evidently not all equal. For instance, the monocytes of the lesions exhibit few signs of death and they probably live longer than neutrophils. Con-

siderations such as these illustrate how complex is the problem of accumulation of bacilli in globus formation.

2. Small Globi

The intracellular position of small globi is not in question. It is the giant globi that are so very difficult to interpret. Because the giant globi have to be considered in possible relation to the smaller ones, it is desirable to describe briefly the smaller globi first. Two types are easily recognizable.

The *cigar packs* are the smallest as well as the most numerous. They are made up of bacilli packed side by side like cigars (or better cigarettes) for their ends do not taper. As indicated in Figure 14, they constitute masses which are not globular any more than a package of cigarettes is globular. The bacilli in the packs are often of the same length so that the lateral limits of the pack, as well as the ends, seen in profile, are straight lines. Quite frequently the pack is tilted with the result that the end meets the sides at obtuse and acute angles. When many packs are observed in a section through a large macrophage, for example, their long axes may lie in many directions. This is shown in the upper right hand corner of Figure 14. Common measurements of cigar packs in sections grade from 3 by 1.2 μ to 3 by 3.6 μ. Globi of this sort can be found in all of the types of cells that have been listed as containing intracellular bacilli, but they are only conspicuous components of the monocyte-histiocyte-macrophage group of cells and their derivatives.

Globi of the second type may be less numerous than the cigar pack globi. They are larger, roughly from 3.7 by 8.4 μ to 6 by 14 μ. In shape they are elongated bodies, somewhat circular in cross section with tapering ends. They somewhat resemble oat seeds and may be called *seed globi.* Because they are not found in so many types of cells as the cigar packs it follows that they are not inevitable developments of the cigar packs any more than all intracellular bacilli accumulate in cigar packs. Most noteworthy among the cells, which may contain small cigar packs but do not exhibit seed globi, are the neutrophilic leukocytes. Seed globi are uncommon in epithelial cells.

Yet no sharp dividing line can be drawn between cigar packs and seed globi. By careful selection globi can be found which appear to represent an unbroken series of transitions from cigar packs to seed globi. With the close association of more bacilli side by side the length of the globus increases because their ends are no longer held in a single plane. On the contrary, some of the bacilli extend beyond the confines of the original cigar pack and increase its length. As a seed globus takes form the packing of the bacilli becomes less close. Fluid accumulates. The clear halo often noted about the cigar packs becomes wider. Stainable material, wrongly called "Schleim" by the Germans because it is not mucus, increases in association with the bacilli. The centers of the larger seed globi appear to be less rich in bacilli than their peripheral parts. Seed globi are well illustrated in Unna's ([13]) unsurpassed colored plates (Figs. 260 and 261).

3. Giant Globi

Unna did not inform us when globi become large enough to be called giants, but it seems to me that those whose diameters, measured in sections, range from 15 to 150 or more μ may be so dignified. They were discovered before the smaller ones and it is from their typical globular form that the cigar packs and seeds take the name "globi," though they themselves are not spherical. Thus Unna ([13]) and Marchoux ([14]) rightly include cigar packs under the heading of globi.

Nevertheless, from the following quotation it appears that Manson-Bahr ([15]) applies the term "globi" to structures different from the cigar packs: "In addition to the bacilli-bearing cells, and increasing in number with the age of the lesion, a number of brown granular bodies, larger and smaller, named 'globi,' are to be seen; these are thought to be cells in which the bacilli have perished and become granular." Clearly the cigar packs are not brown granular bodies, nor are they cells in which the bacilli have necessarily perished and become granular. Gay and Steinbach ([16]), also in describing the bacilli, write that "they occur most characteristically in 'cigar bundle' masses and in colonial clumps that contain enormous numbers of rods. Most characteristic of all is the presence of both intracellular and extracellular gloeal masses, known as 'globi,' which are clumps of bacteria encased in a capsular

material." Since the cigar pack globi are not extracellular gloeal masses of bacteria enclosed in a capsular material, they are apparently not globi in the meaning of Gay and Steinbach.

In tracing the origin of the smaller giant globi, which can be clearly seen to be intracellular, there is no difficulty. By selecting for examination the less dense parts of a nodule the giant cells can be studied more or less individually and their limits definitely established and, within them, the generally rounded giant globi are produced, at least in some cases, by a ballooning of seed globi. The nuclei of the giant cell are displaced usually toward one side, as the nucleus of a fat cell is displaced by enlargement of the contained fat droplet. In both, the growth in size is conditioned much more by the increase in stored material than by increase in volume of cytoplasm in the strict sense of the term. Sharp lines of demarcation are recognizable both between the rounded droplet of fluid containing the organisms and the droplet of fat, and the cytoplasms in the cells that possess them. If the included materials should escape and the cells survive, they would in all likelihood shrink to comparatively insignificant dimensions. Indeed, we do not find the giant globi developing in giant cells possessed of much cytoplasm and many nuclei of fairly uniform size peripherally placed. Such cells, often spoken of as belonging to the Langhans type, are common in the dermal lesions of tuberculoid leprosy where leprosy bacilli are extremely rare. In cutaneous nodular (lepromatous) leprosy, with which we are dealing, they are infrequent and comparatively small. Those that do occur may contain a few bacilli and globi of moderate size, as well as occasional stellate bodies; but it would appear that they are not produced by the dilatation incident upon the formation in them of giant globi in the same manner as the giant cells under consideration, which are, by contrast, the more difficult to identify the larger the size of the contained globus. Figures 17 and 18 illustrate giant globi limited by membranes, the giant cell derivation of which can only be demonstrated in serial sections.

Though it is reasonable to assume, as a working hypothesis, that still larger giant globi are merely enlargements of the smaller ones located in even more swollen giant cells of the same nature, evidence is needed. It is with these really enormous accumulations of bacilli that Denney was chiefly concerned. He regarded them as masses of bacilli enclosed in a membrane of undetermined nature. Principal reliance was placed by him in observations made on mounts of fresh tissues. I have also examined these bodies, obtained from biopsies and mounted in physiological saline plus numerous supravital stains, without much success. When a preparation is made, sufficiently thin to study at high magnification, the giant globi are compressed and their topographical relations with other tissue components are lost. Consequently my study has been based chiefly on sections.

If the membranes that surround the largest globi are not the remains of the investing giant cells, what are they? As indicated at the beginning of this paper, Unna and his followers would have us believe that the

DESCRIPTION OF PLATES

PLATE 36

FIG. 1. Giant globi appear as large vacuoles incompletely filled with deeply stained material. These globi form a layer the outer surface of which extends parallel with the epidermis for the length of the section (2.5 cm.).

Case 29. Formalin-Zenker fixation and Ziehl-Neelsen-hematoxylin stain. X 230.

FIG. 2. Giant globi, containing somewhat less deeply stained material, which is not arranged in the form of a definite layer. Some closely approaches the epidermis.

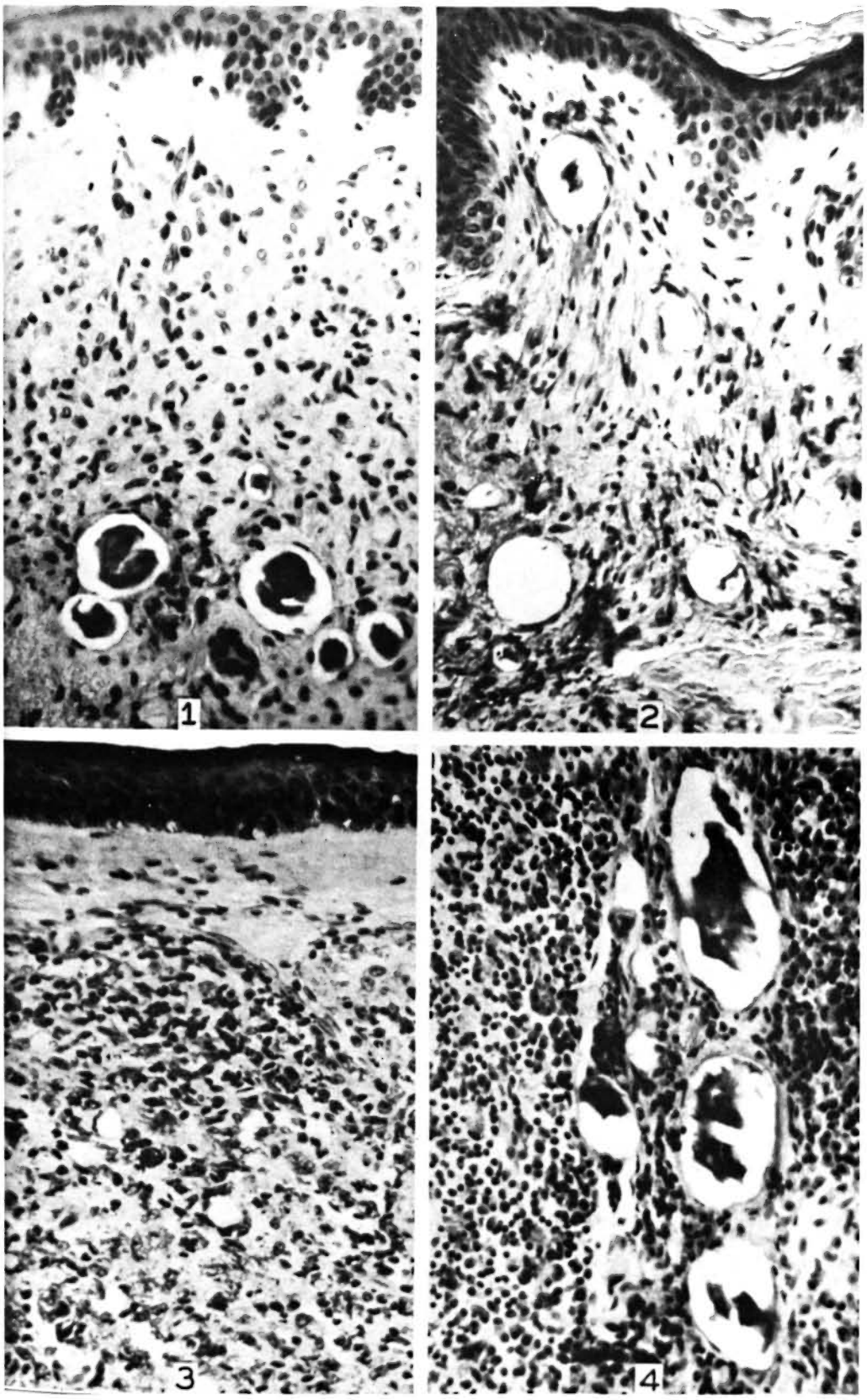
Case 10. Fixation 10 per cent formalin and Ziehl-Neelsen-hematoxylin stain. X 230.

FIG. 3. Section showing ironed out epidermis, Unna's marginal layer with venule and richly cellular lesion beneath. No large globi are seen like those in Figs. 1 and 2.

Case 4. Tissue fixed in 10 per cent formalin and section stained with Ziehl-Neelsen-hematoxylin. X 230.

FIG. 4. Connective tissue septum penetrating into a lymph node with three giant globi in the lymphoid sinus on the right and one on the left. Note that these are much larger than the giant globi represented in the dermis (Figs. 1 and 2).

Case 21. Tissue fixed in formalin-Zenker and section stained with Ziehl-Neelsen-hematoxylin. X 230.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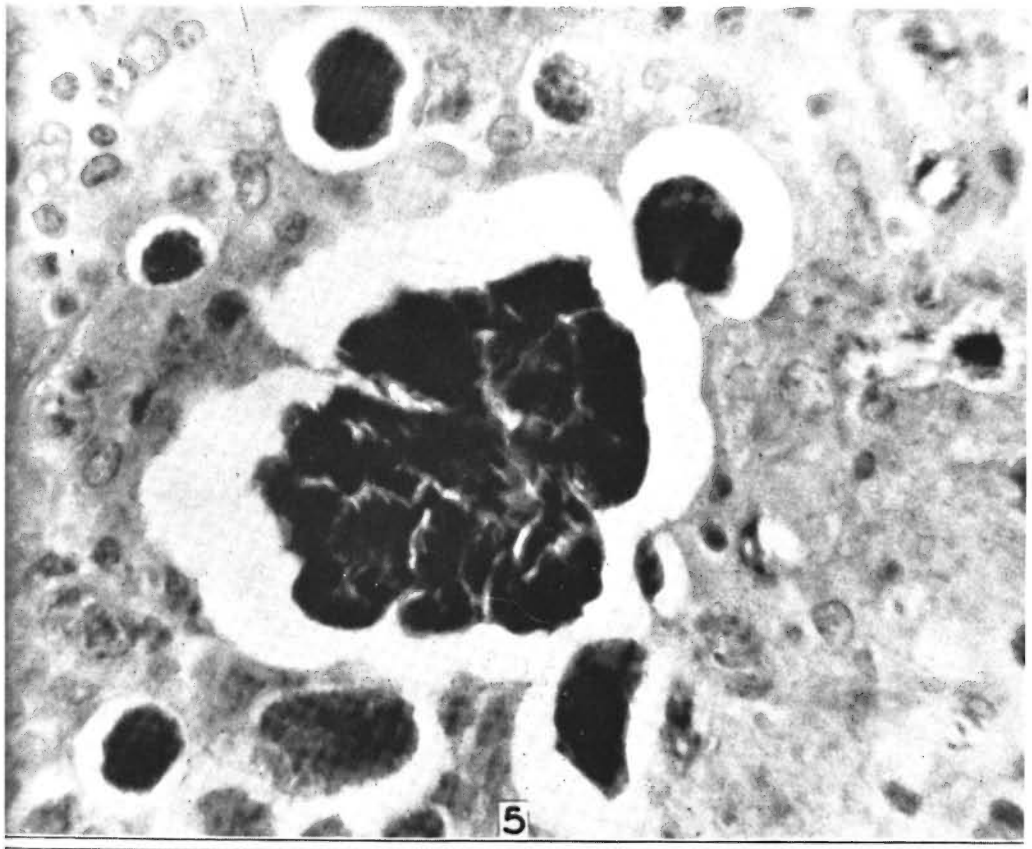

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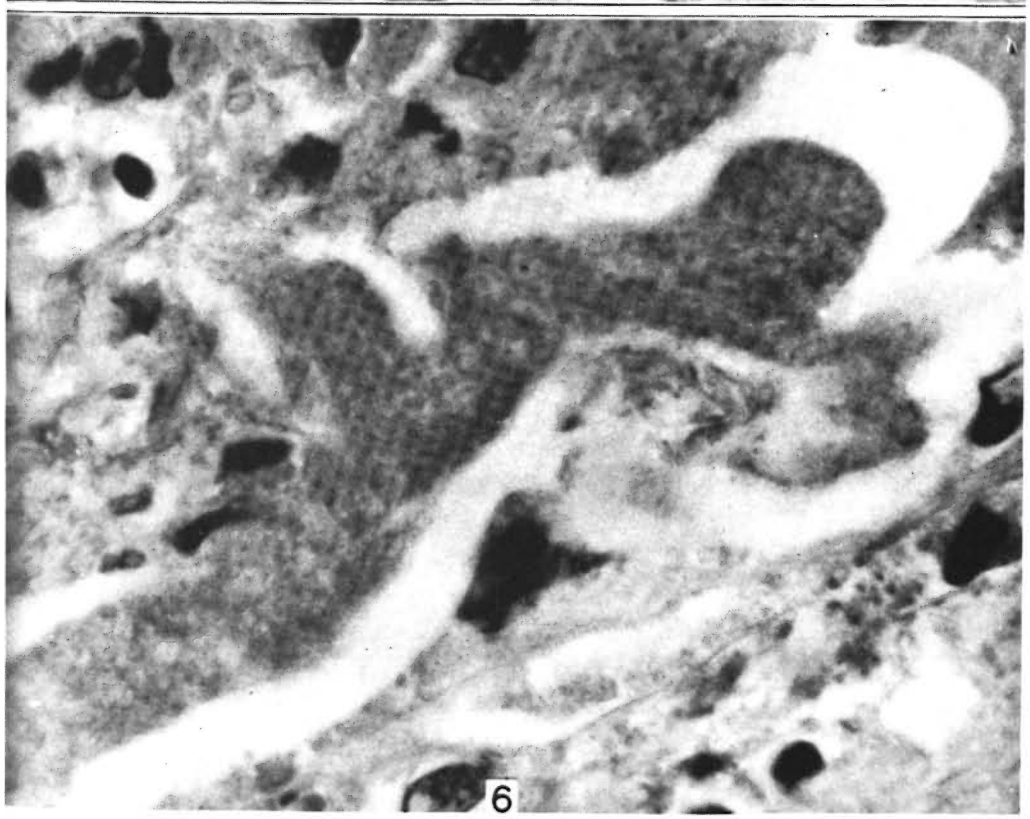

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membranes are the walls of lymphatic vessels and that the giant globi are masses of bacilli which have formed extracellularly in their lumens. The trouble is that we have but few data on the participation of lymphatics in the pathogenesis of the lesions. Leprologists seldom have anything definite to say about them, while the emphasis placed on them by leading pathologists is uneven. MacCallum ([17]) states that the lesions are "... rich in blood vessels and especially in wide lymphatics," and Mallory ([18]) completes his account of leprosy without any mention of lymphatic vessels. Apparently no investigators have demonstrated the lymphatics in leprous nodules by the improved injection method devised by Hudack and McMaster ([19]). Reasons for concluding that giant globi are contained in lymphatic vessels are not convincing.

One often finds (see in Figure 1) that the giant globi are disposed in a layer at a fairly definite and uniform distance from the epidermis. This layer corresponds roughly in position to that of the superficial lymphatic plexus of normal skin as recently studied by Gray ([20]). In other cases, as Gray pointed out, the vessels of normal skin extend into, or better begin in, the dermal papillae, and I find that giant globi may likewise occur in this situation (Fig. 2). In still other cases there are no giant globi near the epidermis (Fig. 3). But correspondence in position of giant globi with the lymphatics of normal skin, or lack of it, means very little either way.

Globus material (bacteria and Schleim) has been frequently reported in varicose spaces which branch and have been taken to be lymphatics. Without seeing the particular specimens an opinion is hardly justified. Some appearances were encountered which may be of the same sort. Figure 6 shows, at high magnification, a large mass of globus material extending from the lower left hand corner of the microphotograph upward and to the right. It is contained within an ill-defined and branching space but the walls of the space are not smooth and endothelium-lined, as would be the case if they were those of a lymphatic vessel. Close examination shows that the globus material, partly invested in fluid, was in this instance free in the tissue and was not limited by any microscopically visible membrane. Further, it was found that this state of affairs obtained near the edge of the section where there were signs of mechanical trauma. The suggestion is advanced that the membranes, enclosing several giant globi, were ruptured and that the contents escaped into the tissue fluid and flowed together.

Examples have been noted of globi resident in spaces, the walls of which project into the lumen in a manner remotely suggestive of valves in a lymphatic vessel. Even though it must be admitted that the structures, if they are valves, may differ materially from normal valves in such a lesion, the differences nevertheless are too great to entertain the theory that we are dealing even with altered values. The projections are neither coated with nucleated endothelial cells nor placed at an acute angle with the wall, like valves. Instead they extend directly into the lumen and appear to be the walls of giant globi which have come together and partly disappeared so that they no longer entirely bridge the gap.

Some evidence can be brought forward that the giant globi observed are not extracellular formations existing within the lumens of lymphatic vessels. They do not exhibit within their investing membranes either the scattered lymphocytes and other cells or the typical coagulum of lymph seen in sections. If, however, they are in the lumens of lymphatic vessels the normal contents of the said vessels must have been in some way banished owing to unusual conditions operative in the lesions.

It has not been possible to establish continuity between the walls of giant globi and the walls of lymphatic vessels. In attempting to do so I first followed rather isolated giant

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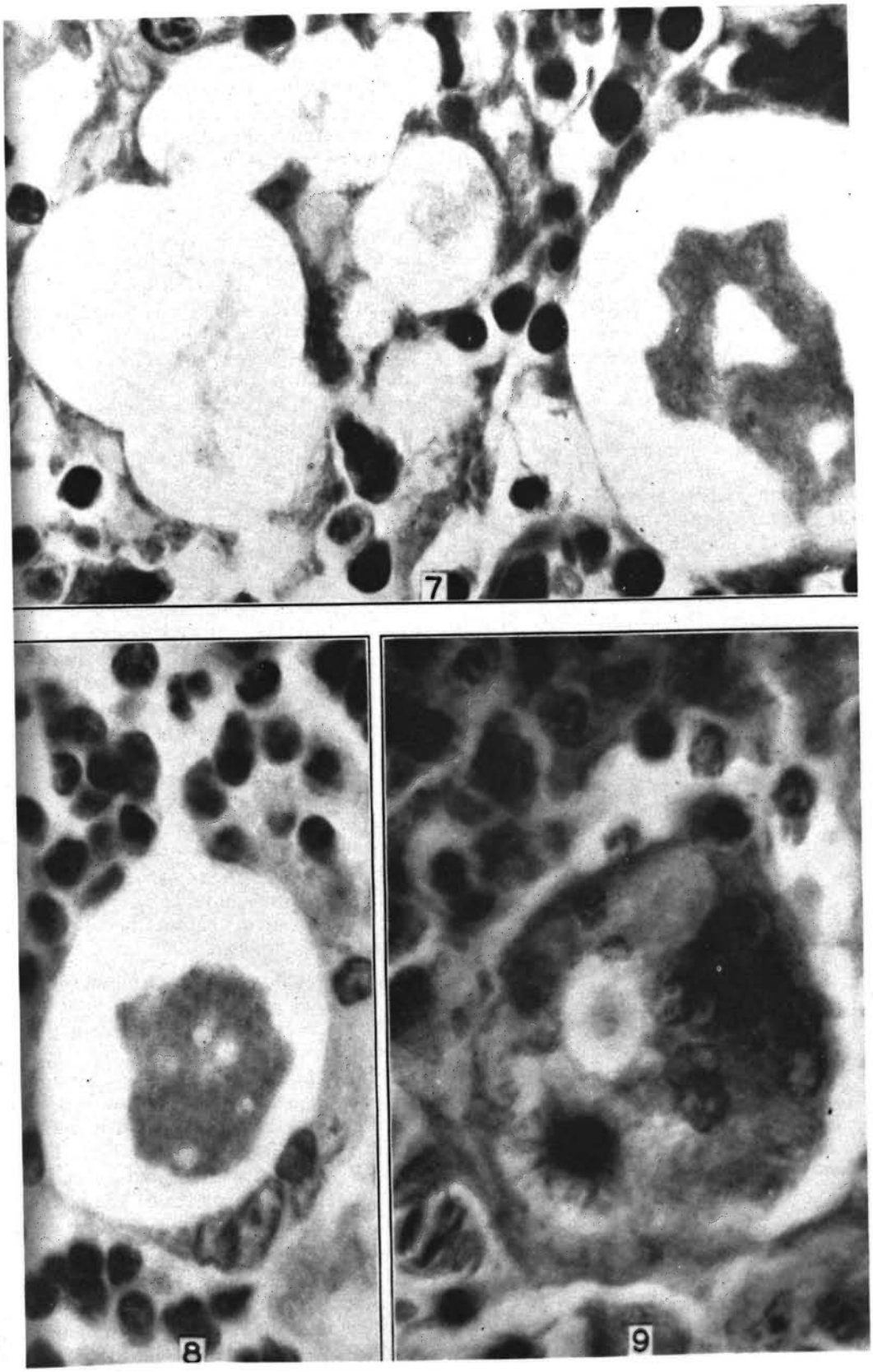
PLATE 37

FIG. 5. Cactus globus in a densely cellular lesion about 11 mm. beneath the epidermis.

Case 6. Fixed in Regaud's fluid and stained by Carpano's method. X 800.

FIG. 6. Globus material escaped from retaining walls and free in tissue fluid. It is not within a lymphatic vessel because it is not limited by a cellular membrane.

Case 7. Tissue fixed in 10 per cent formalin in absolute alcohol and section stained with hematoxylin and eosin. X 1400.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globi, like those represented in Figure 1, from section to section without finding evidence of continuity. Careful study of very thick sections, lightly stained, was not helpful. Numerous reconstructions were then made from serial sections 6 μ thick. In very rare cases one or two delicate thin walled tubes led off from the lumen containing a giant globus. This condition of affairs is illustrated at high magnification in Figure 19. A giant globus is shown in what looks like a clear space, limited by an ill-defined wall, which is incomplete below where the tube leaves and passes to the left. The walls of this tube are smooth and quite definite in the original preparation, but they could not be traced more than about 12 μ. Whether such a tube is a lymphatic capillary or not cannot be stated positively. But, almost always, the walls of the globi are complete, without any outpouching, though it is not unusual for the spaces containing giant globi to unite.

To make reconstructions of giant globi in deeper lesions containing large numbers of them is extraordinarily difficult by reason of the absence of suitable landmarks. However the giant globus in the center of Figure 5 was observed to be in a lumen which communicated peripherally with seven other lumens, each containing a somewhat smaller giant globus. Two openings are represented on the right above and below. Unna observed these formations and designated them *cactus forms* of giant globi. The peripheral giant globi are attached to the central globi like the short thick leaves of a cactus to its branches.

Application of Mallory's aniline blue collagen stain and of Masson's modification of Mallory's stain demonstrates that the walls of these giant globi are not strengthened by collagenic fibers in a manner reminiscent of lymphatic vessels. Weigert's elastic tissue stain brought to light a few elastic tissue fibers but they were not definitely related to the walls of the globi. The absence of a characteristic fibrous backing for the walls and of any vestiges of muscle is evidence that the walls are not those of lymphatic vessels, but does not exclude an interpretation that they may be those of distended lymphatic capillaries. This is, however, improbable because silver impregnations failed to reveal, in the walls, lines of cement substance even remotely comparable to those which outline the component endothelial cells of lymphatic capillaries.

Passing now from cutaneous lesions to those in regional lymph nodes, conditions are found which Unna may have taken into consideration. Here, the globi may be even larger than in the dermis. Compare Figure 4 with Figure 1 taken at the same magnification. In the former a larger and a smaller row of giant globi are shown within penetrating lymphoid sinuses. Examination of the sections at high magnification has, however, shown so many examples of the development of these globi within the cytoplasm of giant cells that an intracellular is much more plausible than an extracellular origin.

Figure 10 represents a section through a giant cell formed from the littoral cells of a peripheral lymphoid sinus. The body of the lymph node is to the right. The lumen of the sinus extends from the upper right margin of the microphotograph downward and to the left. It is much dilated by a giant cell in the cytoplasm of which there are two globi. These may be recognized by the quarter moon shaped masses of bacilli and Schleim, the concavities of which face to the right. The lumen can be traced around the right hand side to the lower border of the giant

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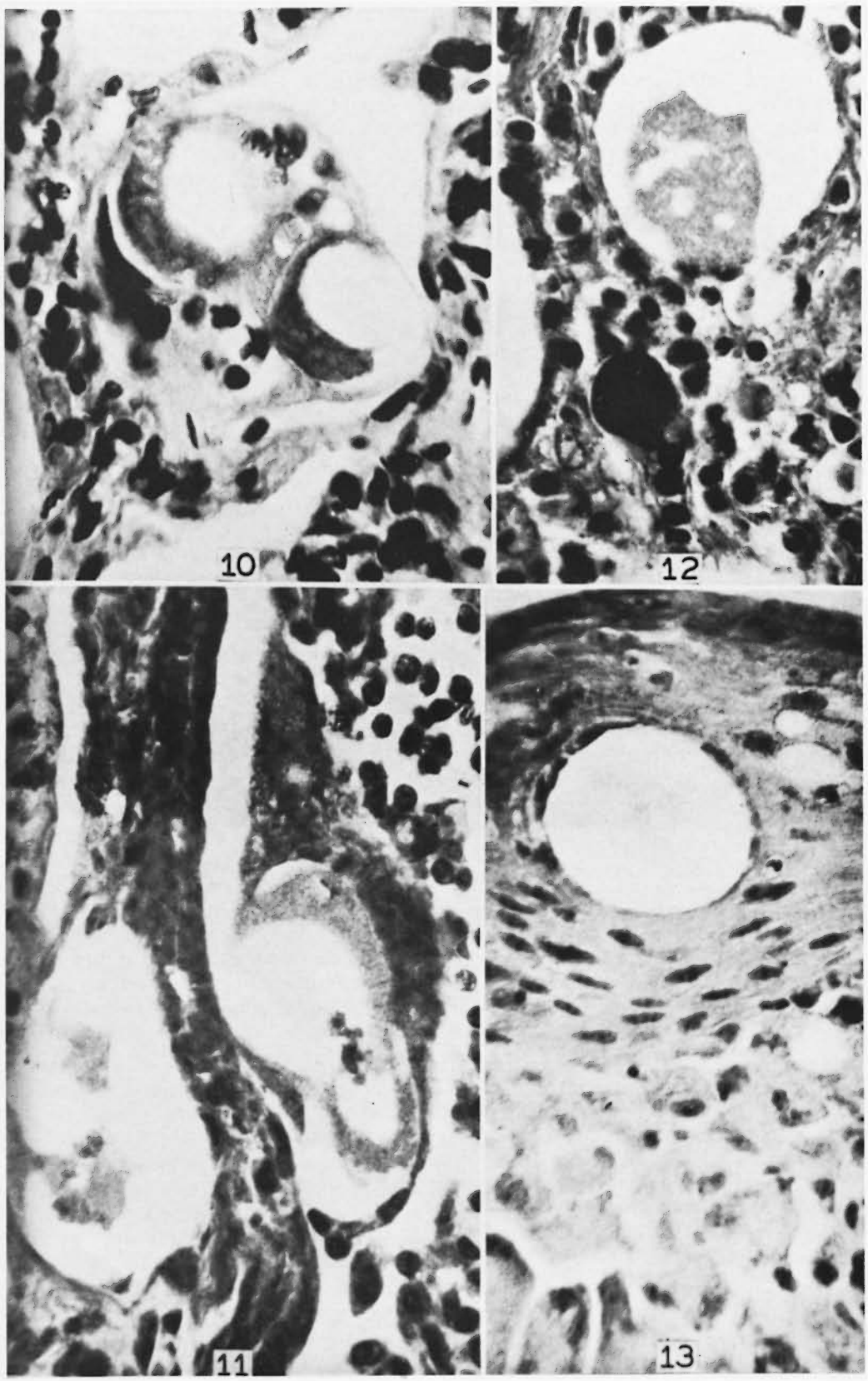
PLATE 38

FIG. 7. Giant globi in cortical substance of a lymph node. The smaller ones to the left are partly confluent and form a cactus globus. The larger one to the right, of which only part is shown, is included within a giant cell, several nuclei of which are seen above.

Case 21. Tissue fixed in formalin-Zenker and section stained with Ziehl-Neelsen-hematoxylin. X 800.

FIG. 8. Giant globus from cortical substance of same lymph node in a section similarly stained. The nuclei of the giant cell containing the globus are clearly shown below and to the right. X 800.

FIG. 9. Giant cell from cortical substance of same lymph node in a section colored by Masson's stain. In the original preparation the stellate body in the lower left part of the giant cell exhibited a bright red stained center with blue radiating strands. Just above the stellate body is a clear area with faintly stained center. This is a beginning giant globus. To the right of it are several nuclei of the giant cell. X 800.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cell. In this section a thin band of tissue intervenes so that the lumen appears interrupted before it extends to the numeral "10," but in neighboring sections there is continuity of the lumen.

Figure 11 is from a section colored by Masson's stain. Extending down the middle and into the substance of the node is a band of collagenic fibers. On either side of the band is a penetrating lymphoid sinus. Applied to the right wall of the sinus on the right is a large giant cell. Within the cytoplasm of this cell is a giant globus, somewhat wider above than below. The wall of the giant cell enclosing the globus is actually complete on all sides but unfortunately the cell wall between the globus and the lumen of the sinus is not shown in the microphotograph.

It is not uncommon to find scattered masses of globus material within the lumens of lymphoid sinuses, as on the left of the collagenic band in Figure 11, but it appears that this is due to the rupture of giant cells containing globi rather than to their formation extracellularly within the lumens.

Only short stretches of afferent and efferent lymphatic vessels outside of the capsules of lymph nodes were examined. Although no giant globi were discovered in them, I am not prepared to say that they would not have been observed had the search been more extended. However, as previously noted, lymphatic vessels observed in association with cutaneous nodules were not found to contain globi and the endothelium of lymphatic vessels is different from, and much less phagocytic than, the littoral cells of the lymphoid sinuses which do form giant cells and intracellular globi. The littoral cells are components of the reticuloendothelial system, whereas the endothelial cells of lymphatic vessels are not.

A survey of the available autopsy specimens shows that globi which are well over 15 μ in diameter, yet not as large as those in the skin and lymph nodes, occur in parts of tissues devoid of lymphatic vessels. They are seen for example in the white and red pulp of the spleen, while lymphatics are restricted to the trabeculae, and in the cortical substance of lymph nodes distant from lymphoid sinuses (Figs. 7 and 8). A few may occur near the centers of hepatic lobules, though the lymphatics are limited to their periphery. Perhaps a more detailed search might reveal giant globi in other alymphatic areas.

Summing up, no evidence has been found that giant globi are formed extracellularly within the lumens of lymphatic vessels. On the contrary a number of observations have been reported which point to the conclusion that they develop intracellularly within giant cells. In many cases they are greatly enlarged seed globi but it is possible that some of them are formed without preliminary orientation of the bacilli either as seed globi

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PLATE 39

FIG. 10. Giant multinucleated cell in a peripheral lymphoid sinus of a lymph node. The cell contains two giant globi with masses of material shaped like crescents in the left hand parts of each. The nuclei of the giant cell are below and to the left of the two giant globi, and this portion of the giant cell is clearly pressed against the wall of the sinus. The upper, right, and part of the right lower margins of the giant cell face the lumen of the sinus. This lumen extends upward and leaves the upper right margin of the figure. In the next section it is continuous with the lumen of the sinus which leaves the lower left margin of the figure. From the same specimen as Fig. 7. ×800.

FIG. 11. Another connective tissue septum penetrating into the substance of a lymph node, comparable with that shown at low magnification in Fig. 4, with lymphoid sinuses containing a giant globus on either side. The globus on the right has been formed by the confluence of two within a giant cell, the right hand margin of which is close to the wall of the sinus. From the same lymph node as Fig. 4, but the section was stained by Masson's method. In the original the dense band of collagenic fibers in the septum was colored blue. ×800.

FIG. 12. A giant globus in the substance of a lymph node distant from both peripheral and penetrating sinuses is represented above and the margin of another on the left. No traces of giant cells are shown. Below is a large spherical lipoid droplet. Same tissue and stain as Fig. 11. ×800.

FIG. 13. A giant globus within stratified epithelium from a case of water buffalo leprosy. This is presented because there is no evidence that the globus is within a giant cell and stratified epithelium is ordinarily both avascular and alymphatic. Tissue fixed in 10 per cent formalin and section stained with Ziehl-Neelsen-hematoxylin. ×800.

or cigar packs. Certainly many cigar packs and seed globi do not transform into giant globi because if they all did giant globi would be ever so much more numerous than they actually are.

The giant cells in the dermal nodules are of the type that have long been regarded [21] as fusions of mononuclear cells (monocytes). In the lymphoid sinuses of lymph nodes they are produced from the littoral cells, while within the cortical substance of lymph nodes they may result from the fusion of cells other than monocytes, perhaps of reticular cells. It is interesting to encounter in the latter "spiculated" or "stellate" bodies (Fig. 9) which resemble those in the dermal giant cells and occur in other conditions as well as in leprosy [18, 22]. The autopsy material available does not permit statements as to the genesis of giant cells possessed of globi in still other parts of the body. Muir and Chatterji [23] do not make a good case for their assertion that "there is reason to believe that the presence of a giant cell in any leprous lesion shows that the part affected is of nerve origin." Neither am I able to agree with them that it is, "... not at all unlikely that the apparent arrangement of the nuclei of giant cells in leprosy is due to the endothelial cells of obliterated capillaries...." It is clear, however, that the cells of the reticuloendothelial system are most involved and that the tendency to fuse is manifested even after giant globi have developed within the said giant cells. This is illustrated in Figure 7 as well as in Figure 5 of a cactus form of giant globus.

4. Significance of Globi

It has been said by Manson-Bahr that the number of "brown granular bodies" (which are giant globi) increases with the age of the lesion, but to supply proof is difficult. While statements are available as to the duration of the disease for all cases from which material was collected, the ages of the individual lesions are not known. In Table II is shown the result of an effort to discover whether there is any relation between the duration of cutaneous nodular leprosy and the incidence of giant globi.

The relative numbers of giant globi are indicated by the number of + signs, but the globi were not systematically counted per unit area of cytoplasm. Estimates were made of their frequency based on the study of many sections. If a larger number of blocks of tissue had been examined a negative report might have been converted into a positive one. Especially in the older individuals, the lesions may have been present for a considerable time before a positive diagnosis was reached. But, speaking generally and admitting the possibility of exceptions, the figures are consistent with the idea that there is an increase in the incidence of giant globi with increase in the duration of the disease.

Obviously the time required for extracellular bacilli to enter the cells, form cigar pack globi, seed globi and giant globi remains to be determined and the ultimate fate of the giant globi requires investigation. The processes may be more rapid in more susceptible persons. It would not be surprising if they are accelerated by fever in the "lepra reaction."

IV. DISCUSSION OF THE INTRACELLULAR BEHAVIOR OF ACID-FAST BACILLI

Since globus formation in human leprosy is essentially an unusual accumulation and arrangement of bacilli within cells, it should be interpreted in perspective, having in mind the intracellular behavior of still other acid-fast bacilli.

It is instructive to contrast rat leprosy and Johne's disease of cattle with human and water buffalo leprosy (Table III). All four have long periods of incubation (or latency), are local infections, usually without fever,

TABLE II. *Incidence of Giant Globi.*

Case number	Age	Duration	Giant globi
	yrs.	*yrs.*	
15	12	4	—
3	42	4	+
5	36	5	++
13	44	7	—
7	30	8	+
14	17	9	—
16	22	9	—
19	23	9	+
4	30	10	++
20	35	16	+++
17	25	18	+
6	35	19	++++

which become chronic and spread slowly, and are caused by acid-fast bacilli which infest mainly cells of the reticuloendothelial system; but in a study of bacteria-cellular relationships the differences are as significant as the similarities. Thus, the human disease is most diversified both in clinical types and in the variety of cells involved, while Johne's disease is most definitely limited to the monocytes and histiocytes.

How far globus formation is conditioned by the bacilli on the one hand, and the cells which they infest on the other, is a question that will for a long time remain unanswered. A few of the many factors which may operate as between the human leprosy bacilli and several varieties of cells in the same host have been mentioned, but the differential be-

TABLE III. *Comparison of Acid-Fast Bacterial Diseases.*

	Human leprosy	Water buffalo leprosy	Rat leprosy	Johne's disease of cattle
Incubation period	5–10 yrs. or longer [24]	Onset not observed	4–6 months	Never less than 1 yr., often over 2 [32]
Age incidence	Chiefly young adults	21 cases apparently all adults [7]	Mostly adults	Chiefly young adults [32]
Sex incidence	In adults reported higher in males, but in children about equal [25]	Data lacking	In question [29]	Data lacking
Course	Several types; in one (tuberculoid) bacilli are infrequent, in another (lepromatous) they are very numerous	One type many bacilli	One type many bacilli	One type many bacilli much emaciation
	Usually very chronic afebrile	Similar	Similar	"As a general rule, the disease is afebrile" [32]
	Febrile type recognized	Febrile type reported but temporary	Not reported	
Lesions	Granulomatous. May be widespread including often nasal mucous membrane; intestine usually not involved [26]	Granulomatous. Skin and occasionally in nasal mucous membrane	Granulomatous, skin, and/or viscera. Occasionally nasal mucous membrane in natural disease, gastrointestinal tract apparently unaffected [29]	Granulomatous. Intestinal mucous membrane
	Nerve involvement may be marked	Not reported	Not reported	Not reported
	Regional lymph nodes	Regional lymph nodes	Regional lymph nodes	Regional lymph nodes
	Fibrosis	Similar	Similar	Fibrosis absent or slight
	Necrosis in large nodules	Similar	Similar	No evidence of necrosis
	Calcification occasional	"Always accompanies necrosis" [7]	Infrequent	Not mentioned

havior of a single sort of bacillus in cells of the same kinds but in different species is even more important. Unhappily there are but few data because when injected into species in which they are not native the bacilli apparently find it very difficult to take hold. But active and sustained multiplication does follow the inoculation of human leprosy bacilli into hamsters (Adler ([27]), Burnet ([28])) and of rat leprosy bacilli into mice of mixed breeds (Sellards and Pinkerton ([31])). In both instances the bacilli became lodged within the same monocyte-histiocyte-macrophage group of cells that they originally inhabited in their natural hosts.

Evidence is scanty on the ability of human leprosy bacilli to form globi in hamsters, as is their custom in humans. Adler made no reference to globus formation, while Burnet, briefly and in passing, reported the presence of globi in smears of subcutaneous tissue which he illustrated by a figure in which nothing resembling a globus can be seen. Both were mainly concerned with transmission.

It has been found that in rat leprosy the bacilli are sometimes arranged in very characteristic rosettes ([33]). These are dense intracytoplasmic masses of bacilli disposed so that they stretch outward, or radiate from a

TABLE III *(Continued)*

	Acid-fast rods and granules	Acid-fast rods and dark red granules + angular forms	Acid-fast rods and granules	Smaller acid-fast rods and granules
	Cultivation?	Attempts –	Cultivation?	Cultivation +
	Chiefly intracellular	Similar	Similar	Similar
	In greatest variety of cells including vascular endothelium	Less than human leprosy in variety of cells not in vascular endothelium	Less than human leprosy in variety of cells. Vascular endothelium "was never found infected" ([30])	In smallest variety of cells
	Form cigar pack, seed and giant globi containing Schleim	"Bundles," and globi even larger than in human leprosy embedded in fat	Rosettes without Schleim or fatty material	Peripheral accumulations without Schleim or fatty material
Bacilli	Fluid accumulation slight about cigar packs and in and about seeds, but marked within investing membrane of giant globi	Even more pronounced within membrane of giant globi	Slight within membrane limiting large rosettes	Not noticeable
	Loss of acid-fastness sometimes seen in bacilli wherever located but particularly in giant globi	No data	No non-acid fast forms found	Not mentioned by Hagan ([32])
	Granulation very common, not all degenerative	Said by Lobel to be degenerative	As in human leprosy	Inconspicuous perhaps owing to small size of organisms
Transmissible to	Hamsters ([27, 28])		Mice of mixed breeds ([31])	Sheep in which species it may occur naturally

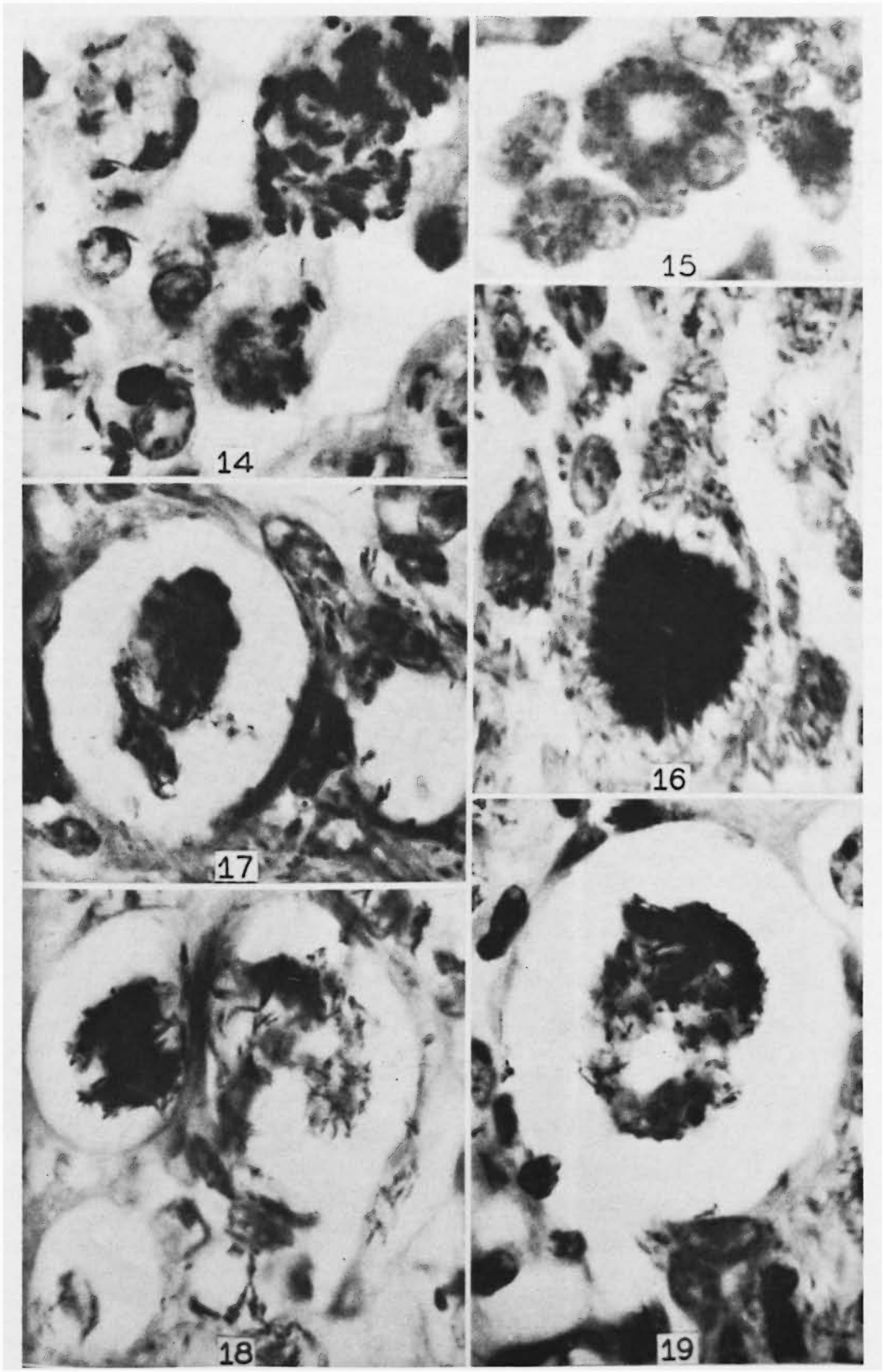
central focus as is represented in Figure 16. Sellards and Pinkerton, likewise primarily interested in transmission, have reported neither the presence nor the absence of rosettes of rat leprosy bacilli in mice. Until, therefore, a purposeful search is made for globi of human leprosy bacilli in hamsters and for rosettes of rat leprosy bacilli in mice —both of course after inoculation with the respective bacilli—it cannot be said whether these formations are conditioned by the bacilli or by species differences in cells belonging to the same category.

Very intriguing is the similarity, described by Lobel, between the globi in water buffalo leprosy and those in human leprosy. He reports the grouping of bacilli "in typical bundles or globi" and adds that the bundle-like arrangement does not suggest "cigar-bundles." Just how close his bundles are to cigar pack globi in human leprosy neither his text nor his illustrations makes clear. The "bacillary globi are," he states, "embedded in fat." This is not the case in human leprosy in which the bacilli are associated with "Schleim" which is not fatty. The globi in water buffalo leprosy occur within multinucleated giant cells, as in human leprosy, while the "great globi" (much larger than in human leprosy) are found "in large cavities," the nature of which he does not specify. The water buffalo material at my disposal is poorly preserved and limited to a few blocks of tissue. I found no evidence of the extracellular development of globi within lymphatic vessels or elsewhere. In one instance a giant globus occurred within a thick layer of stratified epithelium (Fig. 13) in which there are ordinarily no lymphatics. It is to be hoped that Lobel will soon supply further details. At present the resemblance in the bacilli, in the globi they form and in the whole disease reaction, raises the question whether water buffalo leprosy is a disease entity or an infection of water buffalos by human leprosy bacilli.

In Johne's disease the bacilli frequently give rise to discrete rounded colonies in the cytoplasm of the infested cells. Because these resemble more in shape small clumps of heartwater Rickettsiae in endothelial cells ([34]) (and intracellular masses of small organisms occur in a variety of diseases) than they do cigar packs, seed or giant globi in leprosy, it would confuse the issue to call them "globi." In addition I have observed a more unusual accumulation of bacilli in the lesions of Johne's disease, which appears to be at least partly conditioned by the cells in which they live. These monocytes, macrophages or histiocytes, whichever we like to call them, quite frequently show central clear areas about which the bacilli are heaped up in the peripheral cytoplasm. For brevity and convenience the peripheral ring of bacilli may be styled a "peripheral body." One of these is illustrated in a binucleated cell centrally placed in Figure 15. A nucleus can be seen below and to the right, while traces of another are visible in the microphotograph below and to the left. Bacilli are evidently densely packed together in the peripheral cytoplasm leaving a central zone free. This is the appearance in section, but considering the whole cell, the peripheral body, made up of bacilli, is the thick wall of a rather irregular sphere limiting a center containing few if any bacilli.

In the specimens studied the cells containing peripheral bodies were of fairly uniform size and quite numerous, but, since they are yielding structures in a granulomatous lesion, they are not always spherical. The peripheral arrangement of bacilli about the clear central area reminds us strongly of a somewhat similar peripheral arrangement of neutral red granules around a central area in monocytes and macrophages supravitally stained with neutral red. Why neutral red granules become oriented in this way is not known, but it seems to be conditioned by the cytoplasm in which they find themselves. In the central clear area a "central body," or centrosome or diplosome can often be located from which the neutral red granules appear to be held at a distance. Unavailing was a hunt for central bodies in cells possessed of bacilli. However the fixative had not been chosen with this in view. It should have been diluted in accordance with Fry's ([35]) suggestions. Even in the absence of microscopically visible central bodies it is logical to assume that the similar orientation of the bacilli to that of the neutral red granules in cells of the same sorts is occasioned by similar conditions in the cytoplasm.

For some reason the much larger bacilli of human, water buffalo and rat leprosy do not become characteristically arranged in peripheral bodies. Perhaps they are not so subject to the orienting force operating from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the central body area. Occasionally in human leprosy the bacilli are accumulated in the peripheral cytoplasm of cells of the same variety and of approximately the same size as the one shown in Figure 15, but never were they observed to form so dense a mass.

In the present state of our knowledge the cigar, seed and giant globi may be regarded as specific for human leprosy, the rosettes for rat leprosy, and the peripheral bodies for Johne's disease. The bundles of bacilli and globi observed in water buffalo leprosy either indicate a definite disease of water buffalos or an extension of human leprosy to them. Thus far, no clumping of bacilli, even remotely similar, has been reported in human or animal tuberculosis. In all four conditions these peculiar bacillary masses are intracellular formations in cells of the reticuloendothelial system. There is no evidence that any of them develop extracellularly within the lumens of lymphatic vessels.

Although the significance of the formations remains obscure, it is possible that they are not essential stages in the life cycle of the respective acid-fast bacilli. In tuberculoid leprosy bacilli may be of extremely rare occurrence and globi absent. In rat leprosy rosettes are sometimes not seen. Whether they would always be found, if the examination were sufficiently thorough, is doubtful. More data are needed concerning the incidence of peripheral bodies in Johne's disease. Only 21 cases of water buffalo leprosy have been studied by Lobel.

There is no definite correlation between occurrence of the formations and loss in acid-fastness, or granulation, of the respective bacilli. In human leprosy quite large areas of lesion may show loss of acid-fastness of many bacilli both outside and within all varieties of globi. Moreover, in all situations non-acid-fast may exist side by side with acid-fast bacilli. It is unsafe to extend to human leprosy conclusions as to the significance of loss of acid-fastness in tuberculosis ([36]). Rat leprosy is different in so far that there is typically no loss of acid-fastness, while in the lesions of water buffalo leprosy and Johne's disease loss of acid-fastness has not been reported.

Human and rat leprosy are much alike in the frequency of a particular type of granulation of bacilli. In it, the bacilli retain their usual length and diameter but they are made up of granules separated at regular intervals by non-acid-fast materials. This condition is commonly encountered in large stretches of tissue including both globi and rosettes. Apparently it is not an expression of death of the bacilli for it occurs in actively extending lesions. In water buffalo leprosy Lobel refers only to granular degeneration of the bacilli. He says that this phenomenon is more marked than in human leprosy. It may be that he is dealing with a kind of granulation comparable with that which seldom or never

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PLATE 40

FIG. 14. Cigar packs of bacilli within monocytes and macrophages in the center of a large nodule.
Case 17. Tissue fixed in Zenker's fluid less acetic acid and section stained with Ziehl-Neelsen-hematoxylin. ×1400.

FIG. 15. Johne's disease. In the center is a binucleated cell with nuclei below to the left and to the right. They appear lighter than the peripheral cytoplasm charged with bacilli. In the center is a clear area about which the bacilli are arranged in a kind of wide halo.
Section stained with Ziehl-Neelsen-hematoxylin. From material sent by Dr. Hagan. ×1400.

FIG. 16. Rat leprosy. Rosette made up of densely packed radiating bacilli within a multinucleated cell. Comparison with Figs. 14 and 15 shows how different is the arrangement of acid-fast bacilli of different sorts within cells of the same type. Subcutaneous nodule removed from a rat 6 months after injection of emulsion of bacilli.
Tissue fixed in 10 per cent formalin and section stained with Ziehl-Neelsen-hematoxylin. ×1400.

FIG. 17. Two giant globi in a subcutaneous nodule. There are several nuclei in their walls. One, to the lower right of the larger globus, is much elongated, flattened and deeply stained. From the same specimen as Fig. 3 but farther from the epidermis. ×1400.

FIG. 18. Three giant globi from the same specimen as shown in Fig. 17. No nuclei are to be seen in their walls. ×1400.

FIG. 19. A larger giant globus, also from the same specimen. The bacilli are clumped in the center leaving a peripheral zone free. The wall of the globus is continuous to the left, below and to the right, but above these is an opening into a narrow, bent, thin walled tube which can be followed only about 15μ. ×1400.

pervades the whole lesion in human leprosy, is characterized by granules, often of larger diameter than the bacilli and staining more deeply, and which is quite possibly a sign of bacterial degeneration. He reports that the granular degeneration products as well as the rods "are grouped in typical bundles, or globi," but he does not inform us whether the granules occur in the absence of such grouping. If his interpretation be correct the typical bundles are composed at least partly of dead bacilli incapable of multiplication. In Johne's disease granulation of the bacilli is less conspicuous than in the other diseases because the organisms are much smaller. A method, recently devised ([37]) for collecting the bacilli of rat leprosy, free from fibrous and cellular materials, may open the way for more accurate determinations of acid-fastness and granularity.

Large acid-fast droplets occur in the visceral lesions of human leprosy. One of these is represented in black in Figure 12 because it was deeply stained in the original section. Whether they are formed through coalescence of smaller droplets resulting from the degeneration of bacilli remains to be determined. Comparable droplets were not seen in either rat leprosy or Johne's disease. They should be sought in water buffalo leprosy.

Accumulation of fluid about intracellular clumps of bacteria is a common finding in many diseases. In human leprosy this is very marked. It begins about the cigar packs and, in the giant globi, the bacilli are suspended in fluid. Enormous dilatation of the giant cells, in which they reside, is caused more by the increase in fluid than by the number of bacilli. The fluid, when fixed and stained, exhibits the amorphous or granular material, known as "Schleim," which has not been described in water buffalo leprosy and does not occur in rat leprosy or Johne's disease. This fluid accumulation in human leprosy and, according to Lobel, the heaping up of fat in water buffalo leprosy are factors in the great size of the globi. In rat leprosy there is less intracellular fluid accumulation and in Johne's disease little, if any. Since cellular permeability can be increased by Na and K ions and decreased by bivalent cations such as Mg and Ca ([38]), it is possible that the fluid accumulation can be altered experimentally. Spectrum analysis may prove useful ([39]).

V. SUMMARY

1. In dermal lesions the vast majority of bacilli are intracellular. They occur mainly in the cytoplasm of monocytes, macrophages (epithelioid cells), giant cells, "foam" or Virchow cells, vascular endothelial cells and neutrophilic leukocytes. Occasionally they are found in epithelial cells, the endothelial cells of lymphatic vessels, fat cells and the mesenchymatous cells of the perineurium, epineurium and endoneurium. It is doubtful whether they occur in blood and tissue eosinophils and basophils and in cells of the sheath of Schwann. They are not seen in lymphocytes, plasma cells and Russell body cells. In lymph nodes the bacilli occur in those of the above mentioned cells that are present plus reticular, littoral and giant cells. The survey of other tissues, obtainable only at autopsy, is incomplete.

2. The conditions that determine the entry of bacilli into cells are not known. It is not simply a question of contact, or of contending with the cells for the same food supply, nor is it likely that the bacilli actively invade the cells. The number of bacilli in any particular cell is not necessarily a measure of the number taken in. Several factors operate including the rates of destruction and multiplication of bacilli within the cells and the time during which they operate.

3. Within the cytoplasm bacilli may be accumulated without definite order, or as cigar pack, seed or giant globi. The latter may remain solitary, or may partly coalesce, giving rise to the cactus form of giant globus.

4. Cigar packs are made up of bacilli closely pressed together side by side and are generally of about the maximum length of bacilli. They are most noticeable in macrophages, reticular cells and giant cells, but a few bacilli arranged side by side are occasionally encountered in all the other cells that possess bacilli.

5. Seed globi are wider and longer than the cigar packs with ends that tend to taper. They also contain more fluid and some Schleim is present. Since they are not found in all of the cell types that contain cigar packs, it follows that they are not inevitable developments of cigar packs.

6. Giant globi are much larger and more rounded than the seed globi owing to the accumulation of more fluid in which there is also more Schleim. Some of them are en-

largements of seed globi within cells possessed of several nuclei. But, as the giant globi increase in size, the cytoplasm of the multinucleated cells containing them does not increase proportionally in volume, nor is there a parallel increase in the number of their nuclei. Consequently, the cells in question, with progressive distention, look less and less like cells of this category in much the same manner that a fat cell differs from a slim mesenchymatous cell before it accumulates fat. It is for this reason that the investments of the largest giant globi are difficult to identify.

7. Against the concept that giant globi are lodged in lymphatic vessels or capillaries is a good deal of negative evidence. The investing membranes are not made up of endothelium nor are they backed by fibrous tissue as in small lymphatic vessels. It is not feasible to trace continuity between the investment of a giant globus and the wall of a true lymphatic. In the lumen with the globus no lymphocytes or coagulated lymph are seen. No giant globi are observed inside vessels that can be identified as lymphatics. But giant globi of moderate dimensions do occur in tissues ordinarily devoid of lymphatic vessels such as the pulp of the spleen and the central parts of hepatic lobules.

8. Within the substance of lymph nodes, where there are no endothelium-lined lymphatic vessels, giant globi develop within giant cells in the cortical substance distant from the lymphoid sinuses and particularly large ones from within giant cells originating from the littoral cells of the sinus walls. The former sometimes exhibit stellate bodies indistinguishable from those in giant cells of the dermis. The conclusion is advanced that giant globi develop intracellularly in giant cells derived from components of the reticuloendothelial system, monocytes, histiocytes, macrophages, reticular cells and, as representatives of special endothelium, littoral cells of lymphoid sinuses.

9. Finally, comparison of human leprosy with water buffalo leprosy, rat leprosy and Johne's disease is instructive. Like human leprosy, these diseases have long periods of incubation, the lesions are chronic granulomas and the bacilli are chiefly intracellular, many of them living in a kind of symbiosis with the cells. Globi in human leprosy resemble, in some respects, those in water buffalo leprosy. They differ markedly from the rosettes in rat leprosy and the peripheral bodies in Johne's disease. There is no evidence in water buffalo leprosy, rat leprosy or Johne's disease that these distinctive aggregations of organisms are formed extracellularly within the lumens of lymphatics. At least the smaller globi in water buffalo leprosy, and all the rosettes and peripheral bodies in rat leprosy and in Johne's disease, develop intracellularly in cells of the reticuloendothelial system much as the globi do in human leprosy.

RESUMEN

1. En las lesiones dérmicas, la inmensa mayoría de los bacilos son intracelulares. Se localizan principalmente en el citoplasma de los monocitos, macrófagos (células epitelioides), células gigantes, células "espumosas" o de Virchow, células de los endotelios vasculares y en los leucocitos neutrófilos. Sólo ocasionalmente se encuentran bacilos en las células epiteliales, en las células endoteliales de los vasos linfáticos, en las células de grasa y en las células mesenquimatosas del perineuro, epineuro y endoneuro. Está en duda si los bacilos parasitan los eosinófilos y basófilos de la sangre y los tejidos y a las células de la cubierta de Schwann. No se encuentran bacilos en los linfocitos, en las células plasmáticas y en los cuerpos de Rusell. En los ganglios linfáticos, aparte de las células mencionadas, los bacilos se encuentran en las células reticulares, litorales y gigantes. El estudio de otros tejidos es incompleto ya que estos sólo se pueden obtener por autopsia.
2. No se conocen las condiciones que determinan la entrada de los bacilos a las células. No es solamente un fénomeno de contácto o de lucha con las células por nutrientes comunes, como tampoco es muy probable que los bacilos invadan activamente a las células. El número de bacilos en una célula particular, no es necesariamente una medida de su capacidad de ingestión. Intervienen varios factores, incluyendo la velocidad de destrucción y multiplicación de los bacilos dentro de las células y el tiempo en el cual operan tales factores.
3. Dentro del citoplasma, los bacilos pueden acumularse sin un órden definido, como "paquetes de cigarros-puros", o como masas esféricas (globi) pequeñas y gigantes. Estas últimas pueden permanecer aisladas o coalecer parcialmente dando origen estructuras en forma de cactus.
4. Los "paquetes de cigarros-puros" están formados por bacilos estrechamente presionados entre sí lado con lado y tienen aproximada-

mente la máxima longitud de los bacilos. Estas estructuras son más aparentes en macrófagos, células reticulares y células gigantes, aunque pueden encontrarse estructuras similares menos voluminosas en otras células que poseen bacilos.

5. Las "masas-pequeñas" (seed globi) son más anchas y alargadas que los "paquetes de cigarros-puros," y sus extremos tienden a adelgazarse. También contienen más líquido y algo de *Schleim*. Puesto que estas estructuras no se encuentran en todas las células que contienen los "paquetes de cigarros-puros", se deduce que no son la consecuencia inevitable de tales estructuras.
6. Las "masas-gigantes" (giant globi) son mucho más grandes y redondeadas que las "masas-pequeñas", debido a la acumulación de más fluido en el cual también hay más *Schleim*. Algunas de ellas derivan del crecimiento de las "masas-pequeñas" dentro de células multinucleadas. Aún cuando las "masas-gigantes" aumentan de tamaño, el citoplasma de las células multinucleadas que las contienen no aumenta proporcionalmente su volúmen, ni hay un aumento paralelo en el número de sus núcleos. Consecuentemente, las células en cuestión, debido a la progresiva distención que experimentan se parecen cada vez menos a sus células de orígen, del mismo modo en que una célula de grasa difiere de una fina célula mesenquimatosa antes de que ésta acumule grasa. Es esta la razón por la que los límites de las "masas-gigantes" más grandes son difíciles de establecer.
7. Hay muchas evidencias en contra del concepto de que las "masas-gigantes" son formadas y almacenadas en los vasos o capilares linfáticos. Las membranas externas de los mismos no están formadas por endotelio ni tampoco están reforzadas por tejido fibroso como sucede en los vasos linfáticos pequeños. No es fácil trazar alguna continuidad entre los límites externos de las "masas-gigantes" y la paréd de un verdadero linfático. En la luz de los espacios donde se encuentran las masas bacilares no se observan linfocitos o linfa coagulada. No se observan "masas-gigantes" dentro de vasos que puedan identificarse como linfáticos. En tejidos que ordinariamente carecen de vasos linfáticos, como la pulpa del bazo y las partes centrales de los lóbulos hepáticos, se pueden encontrar "masas-gigantes" de dimensiones moderadas.
8. Dentro de la substancia de los ganglios linfáticos, donde no hay vasos linfáticos tapizados con endotelio, las "masas-gigantes" se desarrollan dentro de las células gigantes en la substancia cortical alejada de los senos linfáticos y las "masas-gigantes" particularmente grandes, en las células gigantes que se originan de las células litorales de las paredes de los senos. Algunas veces las células gigantes en la substancia cortical muestran cuerpos con forma de estrella indistinguibles de aquellos en las células gigantes de la dérmis. Se concluye que las "masas-gigantes" se desarrollan dentro de las células gigantes derivadas de componentes del sistema reticuloendotelial, monocitos, histiocitos, macrófagos, células reticulares y de las células litorales de los senos linfoides.
9. Finalmente, es instructiva la comparación de la lepra humana con la lepra del búfalo de agua, la lepra de la rata y la enfermedad de Johne. Como la lepra humana, estas enfermedades tienen prolongados períodos de incubación, las lesiones son granulomas crónicos y los bacilos son fundamentalmente intracelulares, muchos de ellos viviendo en un tipo de simbiosis con las células. Las masas bacilares en la lepra humana semejan, en algunos aspectos, a las de la lepra del búfalo de agua. Difieren, en cambio, de las rosetas en la lepra de las ratas y de los cuerpos periféricos en la enfermedad de Johne. No hay evidencias, en la lepra del búfalo de agua, en la lepra de las ratas o en la enfermedad de Johne, de que estas peculiares agregaciones de los microorganismos se formen extracelularmente en la luz de los linfáticos. Cuando menos, las masas bacilares más pequeñas en la lepra del búfalo de agua, y todas las rosetas y cuerpos periféricos en la lepra de la rata y en la enfermedad de Johne, se desarrollan intracelularmente en las células del sistema reticuloendotelial como sucede en la lepra humana.

RÉSUMÉ

1. Dans les lésions dermiques, les bacilles sont la plupart du temps intracellulaires. On les observe surtout dans le cytoplasme des monocytes, les macrophages (cellules épithélioïdes), les cellules géantes, les cellules spumeuses encore dites cellules de Virchow, les cellules de l'endothelium vasculaire et les leucocytes neutrophiles. A l'occasion, on les trouve dans les cellules épithéliales, de même que dans les cellules endothéliales des vaisseaux lymphatiques, les cellules graisseuses, et les cellules mésenchymateuses du périnèvre, de l'épinèvre et de l'endonèvre. Leur présence dans le sang, ainsi que dans les tissus éosinophiles et basophiles, et dans les cellules de la gaine de Schwann, est douteuse. On ne les trouve pas dans les lymphocytes, ni dans les plasmocytes ou les cellules à corps de Russell. Dans les nodules lymphatiques, les bacilles sont trouvés dans les cellules mentionnées ci-dessus, mais également de plus dans les cellules réticulaires, adjacentes, et géantes. En ce qui concerne les autres tissus, que l'on ne peut obtenir que lors

de prélèvements à l'autopsie, les données sont incomplètes.

2. Les conditions qui président à l'introduction des bacilles dans les cellules ne sont pas connues. Ce n'est pas simplement une question de contact, ou de compétition avec les cellules pour obtenir les mêmes apports nutritifs; ce n'est pas d'avantage que les bacilles envahissent les cellules de manière active. Le nombre de ces bacilles dans une cellule quelconque n'est pas nécessairement une mesure du nombre qui y a pénétré. Plusieurs facteurs interviennent, entre autres les taux de destruction et de multiplication des bacilles à l'intérieur des cellules, de même que le temps pendant lequel ces facteurs sont à l'oeuvre.
3. A l'intérieur du cytoplasme, les bacilles peuvent s'accumuler sans ordre défini, ou bien en paquet de cigares, en chaînette ou sous la forme de globi géants. Cette dernière forme peut rester isolée, ou se fusionner, en donnant naissance à la forme en cactus de globules géants.
4. Les formes dites en paquets de cigares sont constituées de bacilles pressés étroitement les uns contre les autres; leur longueur maximum est habituellement celle des bacilles. On les note surtout dans les macrophages, les cellules réticulaires et les cellules géantes, mais il arrive que quelques bacilles puissent être arrangés côte à côte, et cela peut être observé dans les autres cellules qui hébergent des bacilles.
5. Les globi en chaînette sont plus larges et plus longs que ceux dits en paquets de cigares, avec les extrémités qui tendent à s'amincir. Ils contiennent également plus de liquide, et une certaine quantité de "schleim" est présente. Du fait qu'on ne les trouve pas dans tous les types de cellules qui contiennent des formes en paquets de cigares, on peut conclure qu'elles ne constituent pas une évolution inévitable des paquets de cigares.
6. Les globi géants sont de beaucoup plus grande dimension, et plus arrondis que les globi en chaînette, par suite de l'accumulation de liquide dans lequel on trouve également plus de "schleim". Certains de ces globi constituent en fait des extensions de globi en chaînette, à l'intérieur de cellules qui possèdent plusieurs noyaux. Néanmoins, à mesure que les globi géants augmentent en dimension, le volume du cytoplasme des cellules multinucléées qui les contiennent n'augmente pas proportionnellement; il n'y a pas non plus d'augmentation parallèle dans le nombre de noyaux. En conséquence, ces cellules, affectées d'une distension progressive, ressemblent de moins en moins aux cellules de cette catégorie, le phénomène ressemblant assez à celui que l'on constate dans les cellules graisseuses provenant d'une cellule mésenchymateuse mince, avant que celle-ci n'accumule de la graisse. C'est pour cette raison que les globi géants les plus grands sont difficiles à identifier.
7. Il n'y a pas mal de données qui militent contre le concept qui veut que les globi géants soient logés dans les vaisseaux lymphatiques ou dans les capillaires. Les membranes qui les concernent ne sont pas constituées dans l'endothélium; elles ne sont pas non plus adossées à du tissu fibreux, comme dans les petits vaisseaux lymphatiques. Il n'est pas possible de mettre en évidence une continuité entre les globi géants et la paroi de véritables vaisseaux lymphatiques. Dans la lumière de ces globi, on ne voit ni lymphocytes ni lymphes coagulés. Aucun globus géant n'a été observé à l'intérieur de vaisseaux pouvant être identifiés comme des lymphatiques. Pourtant, les globi géants de dimension modérée peuvent survenir dans les tissus qui sont ordinairement dépourvus de vaisseaux lymphatiques, tels que la pulpe de la rate ou les parties centrales des lobules hépatiques.
8. A l'intérieur de la substance des nodules lymphatiques, là où il n'y a pas de vaisseaux lymphatiques tapissés d'endothélium, des globi géants peuvent se développer à l'intérieur des cellules géantes de la substance corticale, à une certaine distance des sinus lymphatiques, on peut observer de telles cellules aux dimensions particulièrement larges, à l'intérieur des cellules géantes qui proviennent des cellules adjacentes des parois du sinus. On peut parfois y découvrir des formations en étoile, qui ne peuvent etre distinguées de celles que l'on observe dans les cellules géantes du derme. On voudrait en conclure que les globi géants se développent à l'intérieur de cellules géantes provenant des constituants du système réticulo-endothélial, telles que les monocytes, les histiocytes, les macrophages, les cellules réticulaires, et également, représentant un endothélium spécial, les cellules adjacentes des sinus lymphoïdes.
9. Enfin, la comparaison de la lèpre humaine avec la lèpre du buffle d'eau, du rat, et de la maladie de Johne, est fort édifiante. De même que la lèpre, ces diverses maladies ont de longues périodes d'incubation, les lésions étant constituées de granulomes chroniques, et les bacilles étant généralement intracellulaires; la plupart de ces bacilles vivent en une sorte de symbiose avec les cellules. Dans la lèpre humaine, les globi ressemblent à plusieurs égards à ceux que l'on observe dans la lèpre du buffle d'eau. Ils sont cependant fort différents des rosettes que l'on observe dans la lèpre du rat, et des formations périphériques que l'on trouve dans la maladie de Johne. On ne possède aucune preuve dans la lèpre du buffle d'eau,

dans la lèpre du rat, ou dans la maladie de Johne, que ces agregats bien particuliers d'organismes soient formés de manière extra-cellulaire à l'intérieur de la lumière des lymphatiques. Tout au moins, les petits globi observés dans la lepre du buffle d'eau, de même que les rosettes et les corps périphériques de la lèpre du rat et de la maladie de Johne, se développent intracellulairement dans les cellules du système réticulo-endothélial, d'une manière qui ressemble fort à ce que l'on observe pour les globi de la lèpre humaine.

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