LEPROSY; COMMENTS ON IN VITRO BEHAVIOR OF LEPRA AND CERTAIN OTHER ACID-FAST MICRO-ORGANISMS IN PRESENCE OF LEUKOCYTES *

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Several years before actually recognizing the leprosy bacillus Hansen saw and described certain fragile, brownish, elastic masses which Neisser called "globi" and which proved to be collections of the bacilli. Discussion arose concerning the origin of these globular masses, whether they were intracellular (Hansen, Neisser, etc.) or within lymph spaces (Unna). Since then they have received only passing attention. No serious experimental consideration has been given the possibility that they may represent a part of the life cycle of the organism.

The ordinary methods of preparing smears for diagnostic purposes rupture many of the globular masses, freeing the organisms. Sections frequently fail to demonstrate the globi satisfactorily; in thick ones the relationships are obscured, and in thin ones many globi are traumatized (Fig. 1). Tissue juice expressed from a shallow cut in a suitable skin lesion should show disk-like bodies in addition to free and intracellular organisms (Fig. 2).

These bodies, which vary greatly in size but average approximately that of a lymphocyte, consist of a few or many rods seemingly confined within a membrane. Their elastic, fragile nature may sometimes be demonstrated by pressure on the cover glass; if a globus is ruptured the bacillary rods float free. The arrangement of the bacilli in the globus is suggestive of cigarettes loosely filling an inflated

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balloon. They are frequently arranged with their long axes against the restricting membrane, upon which they make no impression.

Occasionally there occur subcutaneous abscesses which contain globi and phagocytosed rods in preponderance over free bacilli. Pus aspirated at intervals will usually show for a time progressive increase in the number and size of the globi. Phagocytosed bacilli apparently continue to multiply until the phagocyte becomes a distended cell wall with a compressed nucleus—the "signet ring" of Hansen.

On the hypothesis that a globus is a colony of bacilli within a phagocyte, multiplication taking place because of suitable pabulum from the protoplasm of the cell and the relative isolation from deleterious influences, the senior author for more than fifteen years has attempted to cause the proliferation of the bacilli by special methods: first, by incubating leprous pus cells suspended in physiologic saline; later, by mixing leukocytes from normal humans with leprous materials; more recently, by adding leprous material to rabbit leukocytes in Tyrode's solution. The evaluation of proliferation of globi would depend, in the absence of successful animal inoculation, on whether globi are pathognomonic. Accordingly, more than fifty cultures of acid-fast bacilli were subjected to the influence of leukocytes.[1]

(a) Leukocytes were obtained by injecting 25 per cent peptone into the pleural cavity of a rabbit, withdrawing the fluid after about twenty-four hours, and diluting it with Tyrode's solution. (b) A suspension of the bacilli was made in Tyrode and filtered through cotton. (c) Glass tubing of 3 mm. bore was cut in 20 cm. lengths, plugged with cotton at both ends, and sterilized with dry heat. At one end, 2.5 cm. from the plug, a constriction was drawn, reducing the bore to about 1.5 mm. At the opposite end, also about 2.5 cm. from the plug, the tube was drawn to capillary size and clipped. The finished pipette was about 50 cm. long. A rubber tube was attached to the cotton-plugged end. (d) The bacillary suspension and the leukocytes were thoroughly mixed in a serum tube and drawn by mouth suction into the pipette, which was then flame-sealed. (e) The pipettes were incubated at 37° C.

In order to examine the contents they were agitated by inverting the pipette and gently tapping the sides; the fluid flowed back and forth, the constriction preventing it from nearing the cotton plug. The capillary end near the seal was clipped with flamed scissors and a droplet was blown onto a slide, spread, dried, and stained. The pipette was again sealed. The examinations were made at twenty-four hour intervals until the leukocytes became disintegrated and the debris overgrown with bacilli.

[1] These bacilli were obtained through the courtesy of Dr. G. W. McCoy, Director, National Institute of Health; Dr. C. W. Duval, School of Medicine, Tulane University; and Dr. M. H. Soule, Hygienic Laboratory, University of Michigan.

The bacilli used are divided into five groups: (1) those universally accepted as pathogenic; (2) those generally accepted as not pathogenic; (3) those isolated from lepers in various parts of the world; (4) those isolated from rat leprosy; and (5) bacilli taken directly from lepers. Of the last there were: (a) tissue juice from nodules obtained by incision and therefore containing considerable blood, and (b) pus aspirated from subcutaneous leprous abscesses. Observations with these two kinds of material have been made repeatedly over a period of years.

1. *Pathogenic bacilli.*—One or more strains of *Mycobacterium tuberculosis hominis, bovis, avium, piscium* and *chelonei.* Their behaviors with rabbit leukocytes were nearly alike. After 30 minutes there was phagocytosis, many of the leukocytes having engulfed one or several bacilli; free bacilli or small clumps were scattered throughout. At 24 hours many of the phagocytes were distended with bacilli, which were growing in a seemingly unorganized manner; not infrequently single rods protruded through the cell wall, indicating its death. Extracellular clumps of bacilli were larger than at first. At 48 hours irregular clumps or colonies predominated; the leukocytes, certainly dead, appeared as débris or so altered as to be not clearly demonstrable (Fig. 3). Further daily observations were without interest, except that when fresh leukocytes were added to the preparation the same cycle of phagocytosis, death of leukocytes and irregularly clumped colonization continued.

2. *Nonpathogenic bacilli.*—One or more strains of *Myco. smegmatis, berolinensis, phlei* and *sterculis.* After 30 minutes phagocytosis was demonstrable, though it seemed less marked than with the tuberculosis group. At 24 hours intracellular growth was not striking, while extracellular colonization was marked. At 48 hours there was no apparent increase in intracellular growth and leukocytic disintegration was marked; extracellular colonization predominated, being particularly extensive in disintegrating leukocytic material.

3. *Cultures from lepers.*—Twenty-five strains represented twenty primary isolations by different leprologists. Some of the cultures proliferated like the pathogenic bacilli, while others behaved like the non-pathogens. Eleven attracted the leukocytes, which phagocytosed single organisms or attached themselves to small clumps; intracelullar proliferation continued until the phagocyte became distended and eventually ruptured, individual organisms often protruding through the cell wall. Fourteen showed no special tendency to phagocytosis

and grew principally extracellularly; when phagocytosed they appeared not to proliferate. The colonization of these bacteria (Fig. 4) bore no resemblance to that of rat or human leprosy bacilli. Periodic addition of fresh leukocytes did not alter their characteristics.

4. *Rat leprosy bacillus.—One strain of Myco. leprae murium* was studied. After 30 minutes there was considerable phagocytosis of single organisms and small clumps. At 24 hours extracellular clumps were much in evidence. Some were compact and spheroidal, indistinguishable from globi. Intracellular inclusions consisted, apparently, of one or more colonies of bacilli distending and distorting the cell, which appeared like the "lepra cell" or Hansen's signet ring. At 48 hours the inclusions seemed much larger but were still within the cell, the nucleus of which was so compressed that it was identified with difficulty. Extracellularly both the irregular, stellate masses and the compact, globus-like bodies had increased in size.

5. *Bacilli from patients.*—After 30 minutes a mixture of leukocytes and leper tissue juice shows considerable phagocytosis, the leucocytes having taken up avidly single organisms and preexisting small globi. However, in subsequent examinations there has not been demonstrated to our satisfaction any increase of either extracellular bacilli or globi or any marked increase in the size of intracellular globi. When additional leukocytes are added there seems to be a diminution in the number of bacterial elements, as would be expected from dilution.

On the other hand, when pus from a leprous abscess is suspended in physiologic saline or Tyrode's solution and incubated, there appears to be for three or four days an increase in the number of globi (Fig. 5), and a coincident increase in their size both within and outside of cells (Fig. 6). When fresh leukocytes are added phagocytosis is noted after 30 minutes. Many of the free rods and smaller globi are engulfed, while the large globi are surrounded, in part or in whole, by numbers of leukocytes. For approximately four days there is evidently an increase in the size of the globi, whether they have been engulfed by leukocytes or simply surrounded by them, but beyond this proliferation does not seem to progress. The globi, intra- and extracellular, remain as such for a long time; a year or more later there are globular masses among the leukocytic débris. If, however, fresh leukocytes are added within four days there is evident continued proliferation of small globi and of bacilli contained within globi, whether within or outside

of cells. This has been repeated through numerous additions of fresh leukocytes, and the numerical relationship of globi and freshly added leukocytes seems to indicate clearly that a progressive increase in the number of globi occurs only so long as free acid-fast rods persist. When through dilution there are few or no such single rods in the suspension, further development of new globi does not seem to occur.

SUMMARY

Acid-fast bacilli in Tyrode's solution have been subjected to rabbits' leukocytes from peptone pleural effusions.

Bacilli of the tuberculosis group proliferated readily in the presence of both living and subsequently dead leukocytes. Phagocytosed bacilli proliferated until the death of the phagocyte. Both intra- and extracellular colonization produced irregular stellate clumps.

Bacilli of the nonpathogenic group proliferated readily, but were not greedily phagocytosed. Colonization was predominantly extracellular, and the colonies were irregular and often stellate.

Of the bacilli cultivated by others from lepers, eleven strains were distinctly attracted to the leukocytes; fourteen were not. With the former, intracellular proliferation continued until eventually the phagocyte was ruptured. The latter grew principally extracellularly, and when phagocytosed appeared not to proliferate rapidly, if at all.

Rat leprosy bacilli were readily phagocytosed, as single rods and small clumps. Within the cell proliferation continued until the phagocyte was distended to the point of rupture, the intracellular growth sometimes being dense and distinctly globular. Extracellular colonies progressively increased in size and some of the dense, spherical masses were indistinguishable from globi.

Leprosy bacilli and globi obtained from an incision in a nodule showed chemotactic affinity with the rabbits' leukocytes, but no proliferation of the single rods or increase in the size of the globi.

Pus from leprous abscesses also underwent phagocytosis. There was no definite increase of free bacilli, but a definite increase in the number and size of globi. Subsequent additions of fresh leukocytes appeared to cause a progressive increase in the size of the globi, and the formation of additional small ones; this formation of new globi apparently ceased when free organisms were no longer present in the suspension.

COMMENT

Globi apparently result from the proliferation of leprosy bacilli within phagocytes, and a globus may apparently arise from a single phagocytosed rod. However, this is not a comprehensive explanation of globus formation, since globi in leprous tissues do not always appear in association with leukocytes. It is not believed that a leukocyte can be distended until, as is occasionally seen, the globus measures 100 microns or more in diameter.

That these globular colonies of bacilli are accidental collections compressed by their own growth into spherical bodies, the shape maintained by cohesion of zooglea or other self-excreted substance, is not tenable since globi may be ruptured mechanically, and organisms that are free and unattached to one another may be seen to escape from the restraining wall.

The nature of the wall of a globus has not been demonstrated by us with any stain. We cannot introduce conclusive evidence that there is a restraining wall, except that a typical globus is a compact, spherical body containing a few or many rods which may be seen floating freely, but which are restrained at the periphery by a definite barrier.

Of the fifty cultures of acid-fast bacilli submitted to living leukocytes in this experiment only one, that from rat leprosy, showed globus formation. While we are of the opinion that this form is a characteristic of rat and human leprosy, it is not felt that the experiment demonstrates that other acid-fast bacilli may not have had at one time the same characteristic. Some of these cultures have been in a laboratory environment for from ten to perhaps thirty years or more.

CONCLUSIONS

1. Of the fifty strains of acid-fast mycobacteria isolated by others and suspended with living leukocytes, only one, that from rat leprosy, showed evidence of globus formation.

2. Acid-fast bacilli contained in "leper juice" suspended with living leukocytes did not evidence proliferation of either free acid-fast rods or globi.

3. Acid-fast bacilli in pus obtained from leprous abscesses evidenced an increase in the number and size of globi, which proliferation was enhanced by the addition of living leukocytes and continued apparently until there were no free organisms in the suspension.

DESCRIPTION OF PLATE

Fig. 1.—Section of leprous nodule. Two medium-sized globi traumatized by the microtome knife. The original spherical shape of the masses may be adduced from the depth of the slices remaining in situ. Free bacilli scattered through the field. About × 1,300.

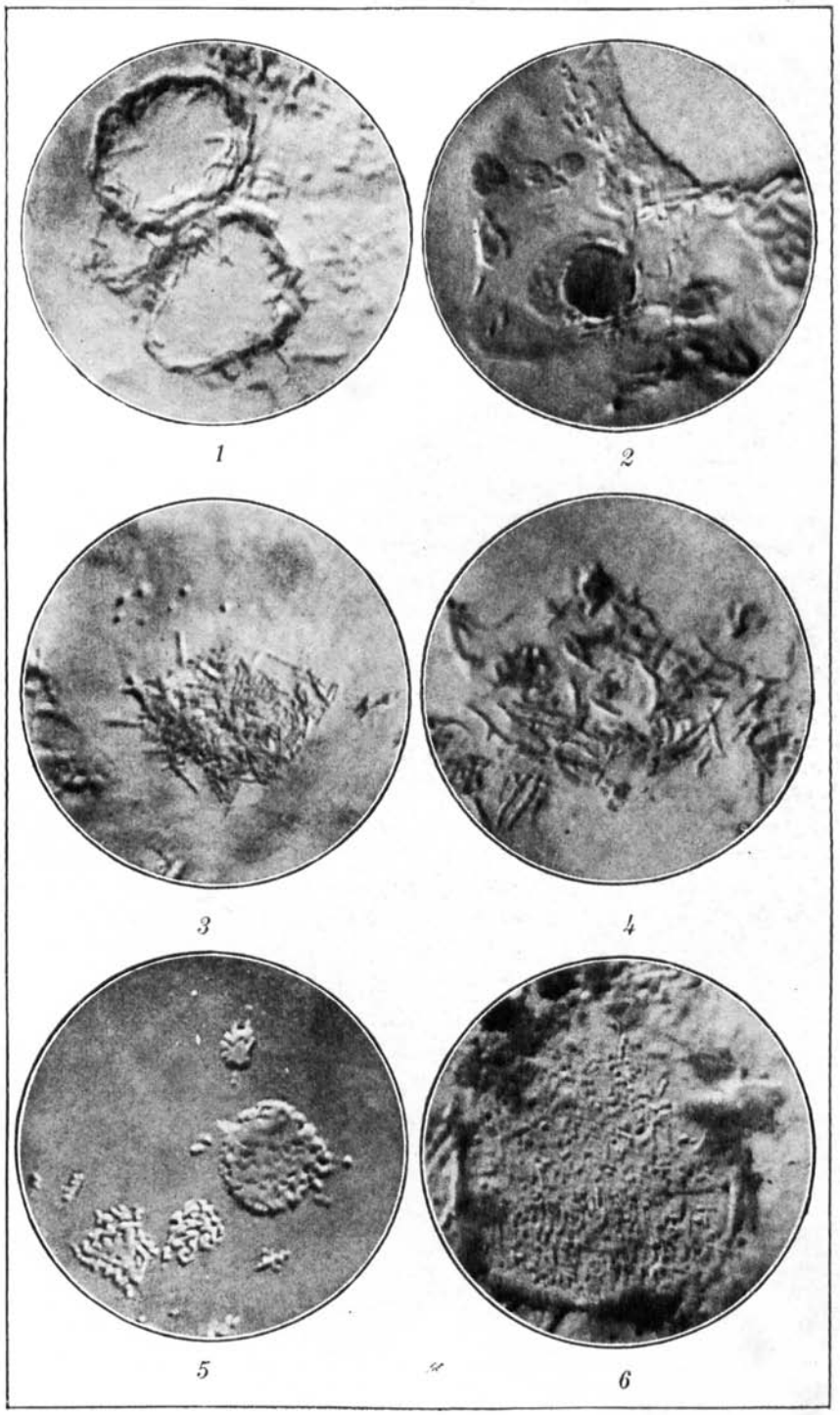
Fig. 2.—Routine, prepared smear of "leper juice". One small intact globus accidentally superimposed on a leukocyte; lymphocytes, a few bacilli and cellular débris scattered throughout the field. About × 1,300.

Fig. 3.—Myco. tuberculosis-heminis. Irregular colonization, rabbit leukocytes remaining as débris. About × 1,300.

Fig. 4.—Myco. leprae Clegg. Irregular colonization; bacilli proliferating in and among rabbit leukocytes. About × 1,300.

Fig. 5.—Myco. leprae, from leprous pus. One almost intact globus and several small ruptured globi. About × 1,300.

Fig. 6.—Myco. leprae, from leprous pus. Large, dense globus almost completely surrounded by rabbit leukocytes. About × 1,300.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