A CONTRIBUTION TO THE CYTOLOGY OF THE LEPROUS LESION.[1]

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(PLATES I.–III.)

SINCE Virchow's description of the tissue changes in leprosy the lesion has been classified as a granuloma. This somewhat elastic term is suggestive of the presence of a new tissue formation taking place as the result of an inflammatory process and characterised by the proliferation, *in situ*, of cells similar in appearance to those seen in granulation tissue. Although the lesions of tuberculosis and leprosy and the gumma of syphilis are the most important as well as the most similar of the conditions classed as granulomata, each lesion presents certain histological peculiarities which at least make a presumptive differential diagnosis possible.

In addition to the comparative absence of necrosis in leprosy, as compared with the other granulomata, the presence of globi or spherical agglomerations of bacilli are diagnostic of the leprosy lesion. Although the significance of the globus and its importance in diagnosis are well appreciated, the greatest difference of opinion exists as to the exact nature and meaning of these bodies. Why such differences of opinion do exist is somewhat difficult to understand, but, since the opposing views are apparently equally well vouched for, the following paper has been prepared, in the hope of explaining both the identity of these bacillary masses, and, in so far as possible, the reason why different theories have been advanced. In addition to bringing forward the result of a careful study of the globus, an effort will be made to describe, more or less completely, the various cytological elements found in the lesions of leprosy, and to trace the histogenesis of certain of the cells.

Two, or perhaps rather four, views are held by different writers regarding the nature of the globi. Controversy chiefly turns upon the situation of the bacilli in relation to the cell bodies. Unna ([1] and [2]) and others claim that the organisms are localised chiefly between the cells, and describe the globus as consisting of a mass of

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more or less disintegrated bacilli lying between the cells, and in particular within the lymph spaces. Upon the other hand, Hansen (1880 [3]) and Neisser (1889 [4]) are of opinion that very few bacilli lie free in the tissues, but that nearly all are to be found within the cells of the lesion. These authors, however, differ in their conception of the constituents of the globus, the former considering them to be collections of bacilli lying in single large cells; while Neisser believes that they are the result of the fusion of smaller cells.

It is unnecessary to attempt to quote, *in extenso*, the ideas of different writers who have published opinions dealing with the nature of the globus. That the question has been considered an important one is evidenced by the extensive amount of work which has been carried on, and the criticisms made by authors holding a certain view on the conclusions of others. Agreeing in general with Neisser and Hansen are Babes (1883 [5] and 1897 [6]), Touton (1886 [7] and 1886 [8]), Doutrelpont (1896 [9]), Rikli (1892 [10]), Storch (1897 [11]), Joseph (1896 [12]), Finger (1896 [13]), Schäffer (1898 [14]), and Lie (1894 [15]). In agreement with Unna, that the globi are clumps of bacilli lying between cells and largely within lymph spaces, are Kühne (1887 [16]), Hermann (1897 [17]), Dohi (1896 [18]), Kanthack (1898 [21]), Bergengrün (1895 [19]), and Gerick (1895 [20]). Sticker states that, in advanced cases at least, the globi are only lymph spaces dilated and filled with bacilli.

Until the publication of several papers by Bergengrün the view favouring the extracellular position of the bacilli in general, and consequently also of the globus, had become more popular. Bergengrün considers the individual globus to be composed of bacilli and coagulated lymph, and states that it lies free in the lymph spaces. He has been extremely active in attempting to popularise his opinions, and has been aided by Unna and his pupils. Bergengrün explains the appearance of multinucleated masses of protoplasm surrounding the bacillary masses to be the result of an endothelial proliferation, and states that the bacteria never enter the protoplasmic mass of the proliferated cells. Both he and Hermann describe and picture irregular and branched collections of bacilli in material expressed from the leprous tubercle as proof of their contention. These branching collections of bacilli are undoubtedly frequently seen; the author, however, considers that their appearance can be better explained than by considering them casts, as it were, of the lymph spaces. The characteristic appearance of these collections of bacilli is that of a spherical mass, the peripheral portion containing well-formed rods arranged more or less concentrically, the central area being sometimes composed of a more homogeneous mass, staining red or grey with Ziehl-Neelsen's method.

The material upon which my own study is based consists of tissue from four cases of tubercular leprosy, and tissue from mice used by Dr. Duval (1910 [22]), and inoculated with his cultures of *B. leprœ*. In three cases specimens were procured during life by operation, and the tissue fixed immediately; in these cases subcutaneous lesions only were examined. In the fourth case the material was derived at autopsy, performed forty-eight hours after death; but despite this length of time the staining properties of the tissue were well

maintained. In this case not only the subcutaneous lesions, but also the effects of the presence of the leprosy bacillus in the tissues of the internal organs, were studied, although no effort will be made in this paper to discuss the distribution of the lesions throughout the body.

Smears were made from the cut surface immediately upon removal of the nodules for the study of exuding material. These preparations were stained for bacilli, and by the methods of Giemsa and May Grünwald for the determination of the cellular elements. The tissues were fixed in Zenker's solution, alcohol, and formalin. Sections were cut after embedding in paraffin, and also with the freezing microtome, for the determination of fat. As a routine Mallory's phosphotungstic acid hæmatin was used. For the identification of special tissues and cells Mallory's acid fuchsin, aniline-blue, and orange G mixture, Weigert's elastic tissue stain and eosin methylene-blue were utilised. For the demonstration of bacilli in the tissues, after overstaining in hæmatoxylin, the sections were placed in carbol-fuchsin for twenty-four hours at a temperature of 23° and decolorised for fifteen seconds in 1 per cent. hydrochloric acid alcohol, the decoloration being completed in 95 per cent. alcohol. This method gives excellent results so far as the demonstration of the bacilli is concerned, and injures but little the cell protoplasm; whereas, if a higher temperature is used the protoplasm is distorted, so that for the purposes of study it is useless. The bacilli can also be well shown in frozen sections by means of scharlach R after formalin fixation; the bacilli, because of their fatty envelope, appear as red stained rods.

Smear preparations from the cut surface of a cutaneous nodule, stained by Giemsa's method, show several different types of cells; many consist of a small dark, granular nucleus surrounded by a small amount of basophilic protoplasm (lymphoid cells). Most of the cells, however, are about the size of an endothelial cell, and have a more or less irregular, oval-shaped, pale-staining protoplasm, which, in many, is distinctly vacuolated (epithelioid). Within the protoplasm of these cells are present irregular-shaped vacuoles.

Within the vacuoles of the epithelioid cells, and diffusely throughout the preparation, is a large amount of granular material and numerous collections of fine rods. Stained with carbol fuchsin, an enormous number of acid-fast rod-shaped organisms are seen. These rods measure from 5 to 7 μ in length, and, for the most part, have distinct granules at either end. Large numbers of them are arranged in pairs. The most striking thing about the picture is the arrangement of the bacilli in large, more or less circular or oval masses, and branching collections measuring from 8 to 80 or more μ in size (Plate I. Fig. 1). Within these masses the bacilli, especially those about the periphery, lie with their long axes parallel to the circumference of the mass. In many instances such masses are seen to be definitely associated with the nuclei of the cells, though the protoplasm is not distinctly demonstrated by the stain used. In association with many of the branching forms are seen numerous areas similar in size and shape to the nuclei of the epithelioid cells.

Bergengrün's conclusions are largely based upon the examination of such material as is here described. Hermann apparently depended entirely upon the appearance of the branched forms mentioned here for his statement that the clumps of bacilli are extracellular. In order to reason that the interlacing branched collections are extracellular, it must be considered that the spaces present represent the situation of cell bodies. Upon careful examination, however, it is determined that the unstained portion does not correspond either in size or shape to the cells of the leproma, but is the space once occupied by the nucleus of the epithelioid cells. It has been shown by Duval's

experiments in the cultivation of the *B. leprœ* that the organisms in the globi are capable of continued multiplication *in vitro*. Although tissues stained after our ordinary methods of fixation, etc., are found to be composed of individual cells, the cell membrane is excessively thin, and we can easily imagine that active bacilli may well grow continuously from one cell to another, and that as the result of injury, such as the cutting of the nodule, the interlacing collections of bacilli are expressed from the cells in which they are lying.

We find, then, that in material expressed from the cut surface of a leprosy nodule certain appearances that are distinctive of this disease are met with. The spherical and sausage-shaped masses of bacilli, as well as the branched collections, must be explained by the study of the sections of tissue themselves. The first tissues here described were stained for the organisms. After the localisation of the bacilli in the tissues has been discussed we will proceed to a confirmation of our findings by means of the study of the cellular elements, making use of special tissue stains.

The tissues examined from Case 4 represent very early tubercle formation. The patient was a man of 45 years of age, with a history of leprosy during five years. The tubercles were situated over the extensor surfaces of both forearms and hands, and varied from 0·2 to 2 cms. in diameter. One large tubercle and a collection of very small nodules were removed for histological study. The skin in the neighbourhood of the small lesions was removed for a considerable area beyond the nodules. In this way an opportunity was given of examining the very early lesions.

The following is a description of sections of tissue, from this case, stained by carbol-fuchsin and counterstained by Löffler's methylene-blue, the sections being cut 18 to 25 μ thick in order to retain the bacilli in the tissue.

Sections cut from the portions containing early nodules, measuring 1 to 2 mm. or less in diameter, show collections of typical epithelioid cells filled with small vacuoles (Plate I. Fig. 2), and very large numbers of giant cells containing globi (Plate I. Fig. 7). There are also numerous spaces containing bacilli about which nuclei are absent, or present only as flattened collections about the periphery (Plate II. Fig. 20). The vacuoles in the epithelioid cells are completely filled with leprosy bacilli, in many forming dense masses in which the identity of the individual bacilli cannot be made out; for the most part, however, the bacilli are seen arranged side by side and in sheaths, lying more or less loosely within the cell cytoplasm. The bacilli toward the periphery of the larger masses stain well; in the central areas of many, however, there is a pale bluish-purple staining granular debris. Not only are the bacilli situated within the epithelioid cells, but are found also within the fibrous tissue cells (Plate I. Fig. 4), especially those in the adventitia of the vessels and within the protoplasm of the endothelial cells. In one or two parts of the section are collections of lymphoid and plasma cells, many of the latter being filled with bacilli. Numerous single scattered bacilli are also seen throughout the connective tissue, and occasionally between the cells. Within the nerves bacilli are seen lying free in the tissues, even though there be no cellular infiltration. Though in many cells an appearance is presented as if the bacilli were situated in the nuclei, it is probable that in reality the bacilli are merely lying in the protoplasm above or below the nucleus. Bacilli are seen not only in those parts of the sections infiltrated with granulomatous cells, but also in the epithelial layer and the zone of connective tissue beneath

the epidermis. Within several moderate-sized blood vessels bacilli are seen, both free in the serum and within the white cells.

In many instances the bacilli have grown from one cell to another, so that although distinct cell outlines can be made out the bacilli form continuous branched collections (Plate I. Fig. 3). Within many of the giant cells are numerous round, red-staining, drop-like bodies, similar to those found developing in plasma cells—Russell bodies —some cells being almost replaced by the protoplasm of these.

The greatest care must be exercised in handling the sections if the bacilli are to remain *in situ*; especially is this the case with those in the large vacuoles in the giant cells, where they appear to be poorly attached and readily break away. In many giant cells the bacilli stain poorly and give up the fuchsin more readily than the clumps in the smaller cells; this observation must be explained apparently as the result of degeneration of the older organisms.

Case 3 typifies the appearance in the older lesions. The material is from a patient dying in an advanced stage of the disease, and the lesions here described were among the oldest in the body.

Sections were prepared from the lobe of the ear and stained for the bacilli in the tissues. All preparations contain enormous numbers of bright red staining bacilli, practically all of which appear as distinct rods and contain fine granules. The majority of the bacilli are lying within the protoplasm of the epithelioid cell, in many forming dense red clumps completely filling the cell body. In many places the groups of bacilli in individual cells can be traced from the protoplasm of one cell into that of another (Plate I. Fig. 3). Although large numbers of the cells are more or less completely filled with clumps of bacilli, many contain vacuoles from which the organisms have apparently been lost in the preparation of the sections. Nowhere do the endothelial cells of distinct blood or lymph spaces contain bacilli in large numbers; an occasional bacillus is, however, seen, apparently lying within the protoplasm of these cells.

It is found, then, that, whether the lesions be early or late, a similar distribution of the bacilli is present, namely, within the tissue cells; in the early lesions a larger number are found extracellularly than in the latter case. This we should not expect to find if the bacilli were able to grow equally well between and within the cells, since there would appear to be less resistance to the formation of clumps between the cells than in the cell protoplasm. The examination of these preparations provides reason for considering that the multiplication of the bacilli is within the cell bodies, and that their presence here is the result of active invasion upon the part of the bacteria rather than of a phagocytosis on the part of the cells themselves, since we find many cells not usually phagocytic, such as the connective-tissue cells and the plasma cells, containing bacilli in their protoplasm.

The following descriptions include the instructive cytological features from a case (1) in which large tubercles had been present for two years. The changes are typical of those taking place in active

lesions of long standing. The patient was a well-nourished white man over whose back, buttocks, and arms, were situated nodules varying in size from 4 to 20 mm.

Section 1 consists of a leprosy nodule 1 cm. in length and 4 mm. deep. The epithelium shows a moderate amount of hyperkeratosis. Beneath the epithelium, and separated from it by a narrow band of connective tissue, is situated a leprosy nodule composed, for the most part, of cells of the epithelioid type, *i.e.* cells containing a moderate-sized, pale blue staining, vesicular nucleus, with a fine chromatin network and a very distinct nucleolus. The protoplasm of many of the cells is homogeneous in appearance and finely granular. Large numbers of the cells, however, show larger or smaller vacuoles; in many instances numerous small vacuoles completely fill the whole cell. In general the protoplasm is more or less oval in form, but in many cases it is definitely spindle shaped. These cells vary very much in size, both with regard to their protoplasm and their nuclei, the nuclei varying from 4 to 8 or 10 μ in diameter, and from 6 to 20 μ in length, the protoplasm measuring, in general, about twice the diameter of the nucleus. In many of the small cells the nucleus takes the stain much more intensely and with a less distinct chromatin arrangement. Here and there are seen collections of deeply staining cells of the lymphoid type, usually arranged in groups. Scattered irregularly throughout the section are comparatively large numbers of typical plasma cells with a distinct "Radkern" appearance. There is a moderate amount of connective-tissue formation throughout the nodule, marking it off into lobules and running through between small collections of cells in the form of fine filaments everywhere.

When examined with the low power the most striking phenomenon is the appearance of large vacuole-like spaces, varying in size from 20 to 80 μ or more in diameter (Plate II.). At first sight many of these vacuoles appear to simulate lymph spaces, one or two nuclei similar in appearance to the endothelial cell being noted about the periphery. Upon closer examination, however, with the oil immersion objective, many are seen to be situated in the protoplasm of the giant cells of the Langhans' type, of which there are comparatively large numbers in the section. Transitional changes can readily be made out from cells with merely one or two small vacuoles filled with a granular debris, to cells in which the nuclei are placed towards one edge of the cell, the protoplasm being replaced by a large vacuole. In other instances these vacuoles have merely the narrowest rim of protoplasm surrounding them, and no evidence of nuclei whatever. Surrounding these cells there is nothing to suggest the presence of either lymphatic or blood spaces. No elastic tissue is seen, nor is the fibrous tissue arranged after the manner of that about small lymphatics. These giant cells are of the definite Langhans' type, consisting of a large amount of somewhat granular protoplasm, and containing from two to twenty or more nuclei, with, for the most part, a horse-shoe shaped arrangement towards one end of the cell. In several of the smaller cells an appearance suggestive of division by amitosis can readily be made out. The nuclei are, for the most part, situated at the opposite end of the cell, away from the vacuoles. The protoplasm of several of the giant cells is extremely irregular in outline, is very granular, and occasionally shows prolongations which spread out between the epithelioid cells in the neighbourhood, their projections measuring 20 to 40 μ or more in length.

Within the protoplasm of many of the giant cells are situated deeply staining, radiating rods or fine cones. These rods do not meet in the centre, but have a circular clear space which in many instances contains a deep staining point, the whole mass measuring 8 to 10 μ in diameter (Plate III. Fig. 23). These bodies are the same as those described by Wolbach ([23]). They

are apparently not parasitic, but are either of the nature of inclusions or represent some change in the structure of the cell as a result of altered activity. The author inclines to the latter alternative as the more likely.

Nowhere in the blood vessels or lymph spaces can any hyperplasia of the lining endothelium be made out. The walls of many of the blood vessels are invaded by the proliferative cells of the leprous lesion (Plate III. Fig. 24). No nerves can be identified in the sections examined. Here and there in the deep layers of the corium are seen round fibrous tissue masses containing a few epithelioid cells, apparently the result of a fibrous tissue replacement of the nerves. No mitotic figures can be seen in any type of cell, although the deep staining of many of the nuclei is evidence of their activity. In places in which there are comparatively large numbers of plasma cells, cells are seen with a less deeply staining protoplasm, although possessing a more or less typical wheel-like nucleus. The appearance of many of the cells is suggestive of a transitional stage between the typical plasma cell and the epithelioid cell. In addition to cells of the Langhans' type, there are many groups of cells which appear to have fused together, forming a large protoplasmic mass in which are several nuclei. In other places, although a distinct limiting membrane can be made out between the cells, the vacuoles seem to communicate with those in neighbouring cells.

Section 2 from Case 1 consists of a nodule measuring 0·4 cm. in thickness, stained with eosin methylene-blue. In addition to the cells described in Section 1, a certain number of cells are demonstrated more distinctly than by the phosphotungstic stain. Throughout the section is a moderate number of eosinophil cells. Here and there, too, is seen an ordinary neutrophilic polymorpho-nuclear leucocyte; in the deeper layers several mast cells are found lying in the fibrous tissue.

Serial sections from the tubercle first described show that many of the vacuoles or globi appearing as if devoid of a cellular wall are in reality part of a large giant cell which has been cut in such a manner that the nuclei, situated as they are at one end of the cell, are not included in the section. When it is remembered that many of these cells measure from 80 to 120 μ or more in diameter, it is easy to see why in any individual section, 10 μ thick, only a small proportion of cells will be cut so as to show that portion containing the nuclei.

A characteristic to which attention has been called by other authors, and which is noted in this lesion, is the fact that the granulomatous process does not invade the corium immediately beneath the epidermis. A narrow but healthy looking band of fibrous tissue is always present between the superficial portion of the leprosy nodule and the basal epithelium. Exactly why this condition is constantly present it is difficult to understand; possibly it is because the leprosy bacillus is unable to flourish under the influence of sunlight, although Duval's experience in the cultivation of the organism proves that a moderate amount of daylight is beneficial. Whatever the cause of this peculiarity, it is of the utmost importance in explaining the absence, except in rare instances, of ulceration, as the epithelium is always able to procure sufficient nourishment, and in addition a certain amount of protection from light, etc., is afforded to the underlying less resistant tissue.

The following section from Case 3 is typical of the lesions found in a case dying of leprosy after a long illness. Practically the whole body was involved in the disease,—the nasal septum, larynx, testicle, liver and spleen, as well as the skin surfaces being attacked. As will be seen, these advanced lesions show an almost complete absence of giant cells and also of globi, showing the relationship of these bodies to the formation of giant cells.

A section prepared from a tubercle from the back of the hand consists of normal epithelium, and a subcutaneous nodule measuring 8 by 5 mm. in size. There is a distinct though narrow layer of rarefied connective tissue between the superficial part of the lesion proper and the epithelium. The lesion consists, largely, of a collection of epithelioid cells, separated here and there, by bands of connective tissue. No giant cells and no large vacuoles are present. The chief cells of the lesion are similar in type to those described elsewhere. They have an oval-shaped or somewhat kidney-shaped vesicular nucleus with a distinct chromatin network. The protoplasm is very pale staining, and contains numerous vacuoles. In some the vacuoles are extremely minute and numerous; in others the whole body of the cell consists of one vacuole with a distinct limiting membrane. In many places these cells appear to be fused together, although no distinct giant cells of the Langhans' type are seen. Several cells of the epithelioid type are seen to be in mitosis, the chromatophores and centrosomes being distinctly shown.

In addition to the dense bands of connective tissue mentioned above, everywhere about the epithelioid cells are seen fine connective-tissue fibrils. A few plasma cells and a moderate number of lymphoid cells are present. In the superficial parts of the lesion there are not a few polymorpho-nuclear leucocytes. In the deepest parts of the lesion several eosinophils and mast cells are seen. The sweat glands appear normal. The walls of many of the small blood vessels are infiltrated by cells similar to those seen throughout the section. One moderate-sized nerve is seen, within which practically no nerve tissue remains, this being almost entirely replaced by epithelioid cells and fibrous tissue.

An interesting and instructive observation from this case was made in the examination of the spleen and the lymph nodes about the neck. In these tissues, although few histologically definite leprosy lesions were found, the cells lining the lymph and blood sinuses were filled with bacilli. Despite the invasion of the endothelium by the organisms, in no instance was any proliferation of these cells noted, nor were giant cells seen (Plate I. Fig. 5).

The following description represents the changes in the tissues in very minute tubercles in sections of skin and subcutaneous tissue from Case 4.

Lying within the corium are numerous small nodules of leprosy cells, varying in size from 1 to 2 mm. or smaller. The site of these collections is chiefly about the sudoriferous glands and about the small blood vessels. The smallest lesions consist mainly of lymphoid and plasma cells, the latter being in the majority. These lesions are situated about minute blood vessels; in the immediate neighbourhood of the vessels a few larger vacuolated cells are seen, together with cells intermediate in type between these epithelioid cells and the plasma cells. The larger nodules are made up almost entirely of epithelioid cells and giant cells. These latter are of two types, some being apparently the result of fusion of the protoplasm of epithelioid cells. These

do not contain large spaces such as those characterising the other cells which are of the Langhans' type. In no instance is any appearance seen suggestive that the giant cells are lying within lymph spaces; none have a basement membrane of either collagenous or elastic tissue fibrils about them. On the other hand, definite blood and lymph spaces, even though extremely minute, are distinctly demonstrated by both elastic tissue and fibrous tissue stains. Within many of the giant cells in the section are seen curious, starfish-like inclusions, similar to those referred to in Case 1, and taking an intense bluish purple stain with phosphotungstic acid hæmatein.

Although it has been noted that the bacilli are present throughout the tissues in places other than those showing cellular lesions, it would appear that in the first instance the reaction against the organisms takes place in the immediate vicinity of the blood vessels. It is, in the early lesion, never endothelial in origin, the endothelial and basement membrane always appearing normal even though bacilli may be present within the endothelial cells. The first change noted is apparently an infiltration in the neighbourhood of the vessels chiefly confined to the adventitia, and thence spreading away from, rather than toward, the lumen of the vessel. Gradually in this area cells of the epithelioid type appear, the exact manner of their development up to the present not being understood.

The study of the early experimental lesions in waltzing mice, produced by intraperitoneal injection of cultures of leprosy bacilli, corroborates the cytological findings in the early human lesions.

The nodules which were situated on the peritoneum over the inferior surface of the spleen and within the mesentery varied in size from 0·2 to 2 mm. in diameter. All lesions contain numbers of leprosy bacilli. The bacilli are situated within cells, with the exception of one or two areas in the larger nodules, where apparently the cells have been destroyed, a poorly staining mass of broken-up protoplasm being seen, together with a few fragmented nuclei. In these areas clumps of bacilli are apparently free in the tissues. The smallest nodules are composed almost entirely of lymphoid and plasma cells, the latter being in marked predominance. The most distinctive forms of plasma cells are situated towards the periphery of the lesions; towards the central area intermediate types are seen between the typical plasma cell and a large, pale-staining cell, approximately 1·5 times the size of a plasma cell, with a vesicular nucleus. In the central part of the largest nodules the cells, although similar in appearance, are much larger, measuring from 16 to 28 μ in diameter. The nuclei of these larger cells are more vesicular and stain more intensely; the chromatin is distinct and fine. The cells of this type are filled with vacuoles, and, as mentioned above, an occasional cell is degenerated. No giant cells are seen in this lesion. Globi are present, but are situated within large mononuclear cells. There is absolutely no proliferation of the peritoneal lining, nor is there any apparent proliferation of the endothelium lining definite blood or lymphatic vessels. Within the spleen, and also within several of the lymph nodes, there are microscopic lesions measuring from 100 to 200 μ in diameter composed almost entirely of cells identical in morphology with the plasma cells, but taking the basic stain less intensely. Many of these cells contain clumps of bacilli.

The constant appearance of either typical plasma cells or cells closely resembling the plasma cell morphologically in all early lesions,

in both man and in the experimental lesions, must be considered as suggestive that the plasma cell is especially potent in the reaction of the organism to the leprosy bacillus. The fact that intermediate types of cells, between the typical plasma cell and the typical epithelioid cell, in the advanced lesion are frequently found, gives reason for believing that these two cells are closely related to one another. As the author has already pointed out (1910 [24]), in a previous publication, the large mononuclear cell found in chronic inflammation in the Fallopian tube is apparently very closely related to the plasma cell. It would appear also that the lymphoid cell, so-called, is also connected, histogenetically, with both of these types of cells.

The fact that mitotic figures are so much more frequently found in the large mononuclear cell—termed for want of a better name, lymphoblast—than in lymphoid, plasma, or epithelioid cells, is reason for believing that this cell is the progenitor of the other types of cell. In short, it is the author's opinion that the lymphoid cell of chronic inflammation, the plasma cell, and the epithelioid cell of granulomatous lesions are closely related to one another, and that their direct ancestor is the large mononuclear form described as the lymphoblast. At present there does not seem to be any means of proof with reference to the origin of this last-mentioned cell. There seems no reason to believe that it is endothelial in origin. It is probable that it develops from the lymphocyte of the blood, as contended by Maximow (1902 [25]), or that it develops from some specially differentiated fixed tissue cell, either the fixed lymphocyte—if this cell have an identity of its own—or the cells in the adventitia of the blood vessels.

Marchand (1901 [26]) has lately suggested that these last-mentioned cells are potent to develop under the influence of irritation into lymphoid and plasma cells.

The presence of acidophilic refractile bodies in the giant cells, as described in Case 4, is probably evidence of the relation of these cells to plasma cells. Although all authors are not unanimous in stating that these bodies occur only in plasma cells, the majority consider that only in those lesions having large numbers of plasma cells are the so-called Russell bodies found. Unna has long been of the opinion that the giant cells are, in reality, developed from plasma cells, although he considers that the plasma cells arise from the fibrous tissue cells of the part.

In order to discuss the question of the endothelial origin of cells, or the localisation in lymphatic spaces of any body such as collections of bacilli, it must be distinctly understood what the author considers an endothelial cell and what he understands by a lymphatic space. The writer agrees with His that endothelial cells are especially differentiated mesoblastic cells which spread throughout the embryo from their original foci of formation by means of budding. The development of the endothelial lined blood spaces occurs before that

of the vessels which are to carry the lymph. As the result of the continuous, constant absorption of fluids and food-stuffs from the blood stream there accumulate in the immediate neighbourhood of the cells fluids and altered food stuffs, the products of cell activity. In certain areas, such as the axilla, these collections of fluid attain to a definite quantity; the necessity therefore arises for some protection of the surrounding tissues from the action or influence of this fluid. As a result, certain mesoblastic cells in this neighbourhood are differentiated to form a water-proof lining; these surround the spaces called lymph spaces, which are filled with the fluid called lymph. In a manner similar to the budding of the endothelium of the blood vessels these lymph spaces spread throughout the body. The author considers that only those cells which develop from endothelial cells should be designated as endothelium, and that only spaces lined by such cells can be considered lymph spaces. Collections of cell fluids may form between individual body cells, but such accumulations do not constitute lymph spaces, even though the fluid within these spaces is very similar to, if not identical with, the lymph.

The reason for this definite statement of the author's conception of lymph spaces must be apparent. If the situation of the bacilli be stated to be within lymph spaces, it must of necessity follow that the spaces in which they lie are lined with endothelium. In no instance in the sections studied have definite lymph spaces lined with endothelium been found to contain bacilli in large numbers, and at no time have proliferated endothelial cells been seen in any situation in which their identity was undoubted. On the other hand, in numerous instances, as in the spleen and lymph nodes of Case 3, although the endothelium was attacked by the leprosy bacilli as was evidenced by the presence of bacilli within their protoplasm, no endothelial proliferation was found. Bergengrün's conception of the giant cells as representing proliferating endothelial cells, must be considered inaccurate, since intermediate appearances are never seen, and such endothelial proliferation, as described by him, is not seen in other vascular lesions. Unna's observations were based upon the study of sections in which the cell protoplasm had been more or less shrunk by means of drying. By this method the bacilli do appear to have an extracellular position. A similar appearance is also presented if the sections are subjected to a high temperature in staining the bacilli; the extremely delicate protoplasm is easily destroyed, and only with the greatest care can its destruction be obviated. If care is taken, however, the extracellular position of the organisms is plainly demonstrated.

SUMMARY.

The histological lesion induced in the human tissues by the presence of the *B. leprœ* consists of collections of lymphoid and

plasma cells, followed by proliferation, *in situ*, of large cells resembling endothelial cells in appearance, having a pale-staining, non-granular protoplasm, and a more or less oval or kidney-shaped nucleus with a distinct chromatin network. The protoplasm of most of these cells is vacuolated, — in some the vacuoles are single and large, completely filling the cell and pushing the nucleus to one side; in others the vacuoles are multiple and small. In thick sections the vacuoles from one cell can, apparently, be traced by direct continuity to another. These vacuoles represent a degenerated area within the cell protoplasm, the result of the action of the bacilli which are usually found within the clear areas.

In the earlier lesions, and especially in those experimentally produced in mice, the plasma and lymphoid cell elements are the predominant cells present. The author is of opinion that lymphoid, plasma, and epithelioid cells are intimately related to one another, the last mentioned being developed in larger numbers in proportion to the duration and intensity of the irritant.

Mast cells are usually present as well as eosinophils. Neutrophil-polymorpho-nuclear leucocytes are very frequently found, and in one case reported in a previous paper (1911 [27]) they were present in such numbers as to form distinct abscesses, although no organism other than the leprosy bacillus was present in the lesion.

There are also usually present, especially in the early lesions, giant cells of the Langhans' type, having a large amount of irregular granular protoplasm and containing numerous nuclei which are usually situated toward one end of the cell and frequently arranged in a horse-shoe shaped manner. Division by amitosis in these cells is frequently seen. In cells of the epithelioid type karyokinetic figures are occasionally found. In addition to these distinct giant cells, irregular agglomerations of cells of the epithelioid type occur. The difference in the arrangement of the nuclei in such cells and the absence of granular protoplasm make their differentiation from the true giant cells easy.

A feature differentiating the giant cell of the leprous lesion from that found in tuberculosis is the presence in the great majority of cells of round vacuole-like spaces when stained with a simple stain. These spaces vary in shape from spherical to sausage-shaped bodies, and vary in size from 4 to 100 or more μ in diameter. The nuclei are situated, as a rule, at the opposite end of the cell from that occupied by the vacuole. In the advanced cases they are spread out, forming a flattened, narrow periphery to the vacuole, the whole cell then resembling in appearance a dilated lymphatic space (Plate II. Fig. 20). In sections 8 to 10 μ thick many of the vacuoles are present not showing any nucleolated cell body, although in all cases there is a narrow margin of granular protoplasm. This appearance is the result of the cutting of the cells in such a way that the

nuclei are not brought into the section. That this is the case can be proved by means of serial sections. A certain number of cells of this nature appear to have been completely replaced by the vacuole-like body.

Practically all the bacilli seen in the section are situated within the protoplasm of the tissue cells. Not only are they present in the two types just described, but they are also found lying within lymphoid, plasma, and connective-tissue cells and the endothelium lining the lymph and blood spaces. Although bacilli are not infrequently found in connective-tissue cells in the superficial layers of the corium and in the fat cells, they are much more constantly present in the cells of the adventitia of the vessels. In many instances the masses of bacilli are continuous from one cell to another, forming in this way irregular branching bodies.

A narrow zone of normal looking corium is always present immediately beneath the epidermis. Although this zone is free from inflammatory cells bacilli are not infrequently found.

Material scraped from the surface of an incised tubercle always contains enormous numbers of the leprosy bacilli. A certain number of scattered organisms are usually present, though the greater number are in the form of spherical masses (globi) and irregular branched collections. As a rule no cell bodies or nuclei are noted in direct relationship to the masses of bacilli. The shape of the cell body, however, and the spaces occupied by the nucleus of the cells from which the branched forms are derived can always be made out. That only a small number of nuclei and cell bodies is found is explained readily by the study of the tissues themselves. Everywhere between the epithelioid cells are found collagenous fibrils acting as a support to the cell protoplasm. The epithelioid cells, and more particularly the giant cells, have an extremely irregular outline, numerous prolongations of the protoplasm protruding everywhere between neighbouring cells. In view of this fact, and the fact that the cell membrane, as demonstrated in stained preparations, is extremely thin and the vacuoles filled with bacilli are proportionately large, it is easy to understand why the bacilli are thrown off from the cells as a result of the trauma of incision; whereas the cells themselves, owing to their projections, are more firmly attached and do not exude so readily.

I wish to express my thanks to Dr. C. W. Duval for the opportunity to avail myself of the tissues from mice inoculated with his cultures. For help in procuring material at the Leper Home at Bruns I am indebted to Dr. Ralph Hopkins of New Orleans.

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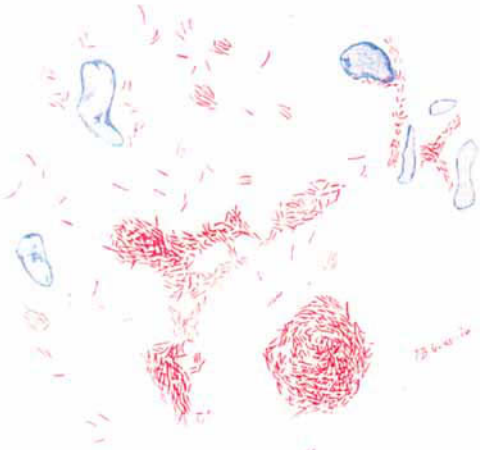


FIG. 1.

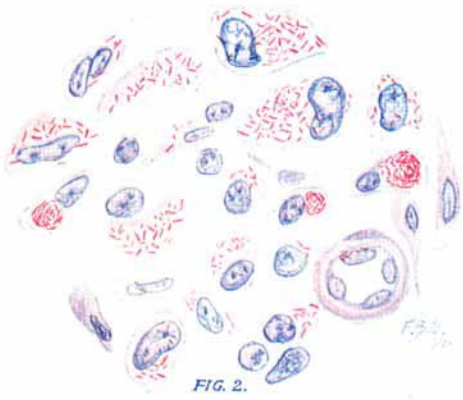


FIG. 2.

FIG. 3.

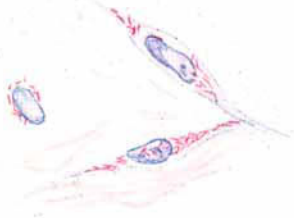


FIG. 4.

FIG. 5.

FIG. 6.

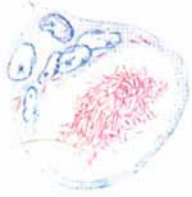


FIG. 7.

FIG. 8.

FIG. 10.

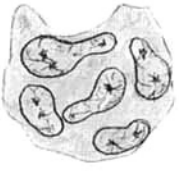


FIG. 9.

FIG. 11

FIG. 12.

FIG. 13

FIG. 14.

FIG. 15.

FIG. 16

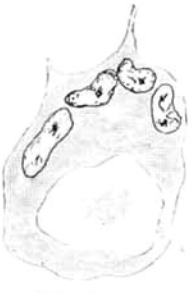


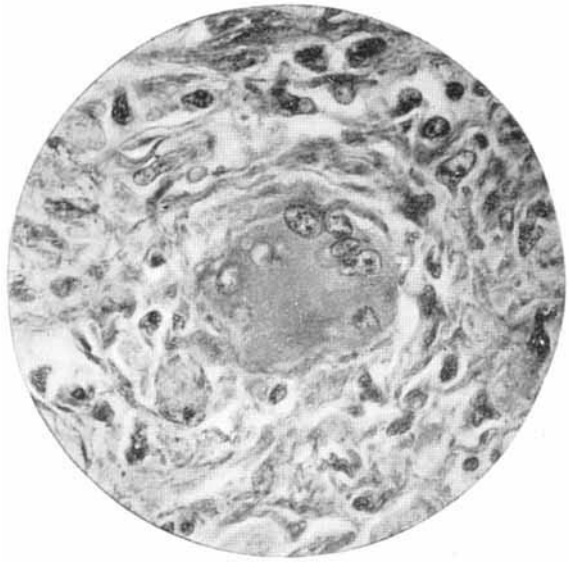
FIG. 18.

FIG. 17

FIG. 19.

FIG. 21.

FIG. 20.



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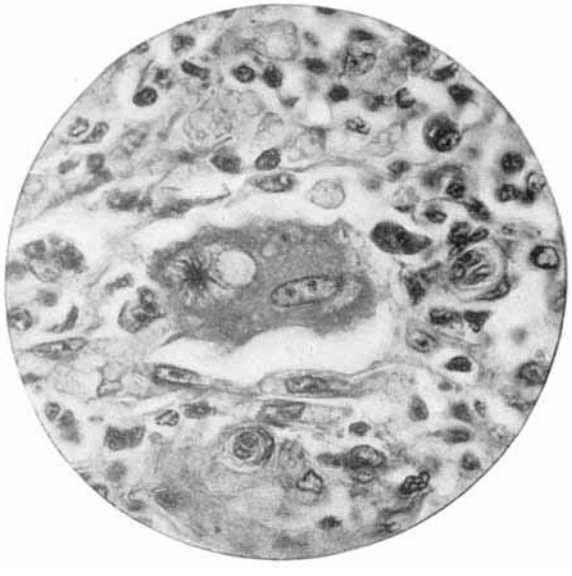

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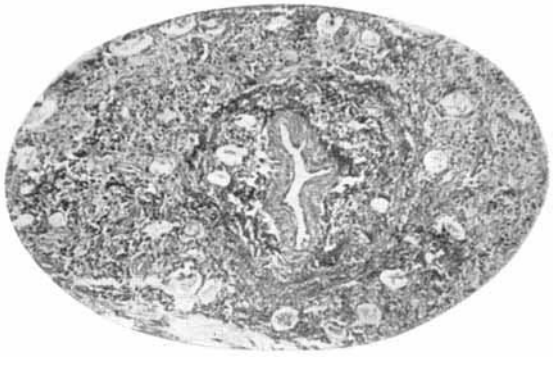

FIG. 24.

DESCRIPTION OF PLATES I. TO III.

PLATE I.

(Drawings.—Magnification about 1500 diameters.)

FIG. 1.—Smear preparations of exuding material from incised leprosy nodule of the skin, stained with carbol fuchsin, counterstained with Löffler's blue.

FIG. 2.—Section of skin nodule, stained for bacilli, showing intracellular position of bacilli, —some diffusely throughout the protoplasm, others in the form of small globi.

FIG. 3.—Collection of cells from a cutaneous nodule stained for bacilli, showing extension of bacillary masses from cell to cell.

FIG. 4.—Connective-tissue cells, showing bacilli lying within protoplasm of cells.

FIG. 5.—Cells lining sinus in spleen. Note absence of proliferation of endothelium and extension of bacilli in continuous masses from cell to cell. Case 3.

FIG. 6.—Giant cell containing a few bacilli.

FIG. 7.—Giant cell containing a large collection of bacilli (globus).

PLATE II.

FIGS. 8-21.—Types of giant cells found in early lesions; phosphotungstic acid hæmatein stain. Note gradual development of lymphatic vessel-like spaces. (Drawing magnification about 1500 diameters.)

PLATE III.

FIG. 22.—Microphotograph of deep portion of early lesion showing large number of spaces. (Magnification 100 diameters.)

FIG. 23.—Microphotograph of giant cells containing no vacuole, surrounded by epithelioid and plasma cells. (Magnification 1500 diameters.)

FIG. 24.—Giant cell from leprous nodule with small vacuole and star-shaped body described in Case 1. (Magnification 1500 diameters.)