THE ORIGIN OF THE LEPRA CELL.

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PLATE 4.

(Received for publication, October 19, 1925.)

One of the most striking features of the anatomical changes which develop in leprosy is the occurrence in the lesion of great numbers of large phagocytic cells filled with bacilli. These cells were first noted by Virchow,[1] and were given the name of "lepra cells" by him. Later investigation has shown that they are characteristic of the disease, the leprous nodule being composed almost entirely of them with a varying admixture of fibroblasts and plasma cells or lymphocytes. In this regard at least they may be considered analogous to the epithelioid cell of the tubercle.

The morphology of these cells is remarkably constant. They vary in size from that of a lymphocyte to several times this diameter. Their protoplasm, which forms the greater part of the cell, is filled with vacuoles, and contains enormous numbers of lepra bacilli. The nucleus is usually single and is as a rule pressed to one side by the vacuoles and bacilli that crowd the cell body.

There is still considerable controversy as to the origin of these cells.

Some have even denied that they are cells but interpret them as lymphatics plugged with bacilli or clumps of bacilli embedded in a jelly-like substance. According to Neisser[2] and Marchoux[3] they are of hematogenous origin, and are derived from lymphocytes which have wandered out into the infected tissues from the circulating blood. They therefore consider them examples of Metchnikoff's macrophages. Baumgarten[4] and Philippson,[5] on the other hand, describe

[1] Virchow, R., Die krankhaften Geschwülste, 1864–65, ii, 514 *et seq.*

[2] Neisser, A., *Virchows Arch. path. Anat.*, 1881, lxxxiv, 514.

[3] Marchoux, E., and Sorel, F., *Ann. Inst. Pasteur*, 1912, xxvi, 675.

[4] Baumgarten, P., *Berl. klin. Woch.*, 1888, xxv, 217.

[5] Philippson, L., *Virchows Arch. path. Anat.*, 1893, cxxxii, 229.

them as originating from the fibroblasts and fixed connective tissue cells of the tissue involved. The problem in regard to their histogenesis is therefore the same as exists with regard to the analogous epithelioid cells of the tubercle. Purely morphological study led to no certain decision as to the origin of these cells and it was only the introduction of a method of functional differentiation by means of vital staining that objective evidence was obtained. By this means Oppenheimer,[6] Goldmann,[7] and others showed conclusively that the epithelioid cells of the tubercle are derived at least in part from certain tissue cells; namely, from the histiocyte or reticulo-endothelial cells.

Although the inoculation of animals with lepra bacilli produces only doubtful reactions, material for an experimental study of the question is available in the spontaneous leprosy that occurs in rats. The relation between the two diseases, that of man and that of the rat, is certainly a very close one. The microscopic appearance of the lesions is in all essentials identical, the lepra cells in particular being equally well developed in the infection of the rat as in that of man. Moreover, several investigators, Mezincescu,[8] Schmitt,[9] and Bayon,[10] have found evidence by means of complement fixation tests which indicates a close relationship between the organisms found in the two diseases. Bayon, in fact, considers them to be identical.

Through the kindness of Dr. N. E. Wayson, of the United States Public Health Service, we obtained a wild rat in which the disease was well developed and have applied the method of vital staining to a study of the origin of the lepra cells in animals inoculated from this source.

Methods.

Six white rats were inoculated intraperitoneally May 23, 1924, with an emulsion of the subcutaneous leprous infiltrations from the spontaneous case. Examination had shown the material to be loaded with acid-fast bacilli. Two of the animals died of intercurrent infections during the next few months, and the remaining four showed no external evidence of the disease after 6 months. At 10 months two rats were given a subcutaneous injection of 2 cc. of 1 per cent aqueous trypan blue and the injection was repeated twice at intervals of 6 days. At the end of

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[6] Oppenheimer, R., *Virchows Arch. path. Anat.*, 1908, cxciv, suppl., 254.

[7] Goldmann, E., Neue Untersuchungen über die äussere and innere Sekretion des gesunden und kranken Organismus im Lichte der "vitalen Färbung," Tübingen, 1912, 67.

[8] Mezincescu, D., *Compt. rend. Soc. biol.*, 1908, lxiv, 514.

[9] Schmitt, L. S., *Univ. Calif. Pub. Path.*, 1911, ii, 29.

[10] Bayon, H., *Brit. Med. J.*, 1912, ii, 1191.

the experiment they were stained a deep blue but otherwise appeared normal. They were then killed and fixed *in toto* in 10 per cent neutral formalin.

Dissection of the animals showed in the inguinal regions and extending over the lower abdomen a diffuse infiltration with soft granulation tissue. Smears of this tissue showed enormous numbers of acid-fast bacilli. No lesions were noted in the internal viscera.

The remaining two rats were stained in the same way 11 months following their inoculation and were then killed. The lesions found in them were of the same nature as in the earlier experiments except that the process was rather more widespread in the subcutaneous tissue and that small nodules were found in the liver and lungs of one animal. Material from the different animals was then embedded in paraffin and sections stained with carmine, hematoxylin and eosin, Van Gieson's mixture, the Giemsa method, and with carbolfuchsin for the bacilli.

EXPERIMENTAL FINDINGS.

The lesions found in all the animals are substantially the same and for this reason only a general description of them is given. The essential process in all the tissues is the development of nodules of lepra cells followed by a late necrosis. These nodules are of the same character and the manner of their formation is the same whether in the subcutaneous tissues or in such internal organs as the liver and lungs. The nodule consists of an accumulation of lepra cells (Fig. 1). These are large oval cells, except where they are compressed by crowding together, whose abundant protoplasm is filled with round vacuoles. In some instances these vacuoles are so numerous that there is little actual protoplasm remaining, the cell body having the appearance of a foam. The nucleus of the cell is small and oval and is compressed and displaced to one side by the vacuoles which crowd the cell body. If the section has been stained with carbolfuchsin and decolorized with acid alcohol the significance of the vacuolization and distension of the protoplasm is seen. The lepra cell is so filled with acid-fast bacilli that all details, except the compressed nucleus, are obscured. The whole nodule appears to be made up of clusters of bacilli and it is only with some difficulty that the cellular nature of the individual clumps becomes evident. Not all the bacilli are intracellular however. Many lie free in the tissue, liberated apparently by the rupture of the distended lepra cells. These in turn are taken up by phagocytic cells and as intracellular proliferation of them progresses this

phagocyte is gradually transformed into the typical lepra cell and forms part of the nodule.

This process of growth of the nodule by the approximation of new cells can be quite clearly followed at its periphery and there the genesis of the lepra cell is clearly evident in the vitally stained animal.

In the subcutaneous lesions the nodules lie scattered through the loose alveolar connective tissue that lies over the fascia of the muscle. On examining this tissue one is immediately struck by the tremendous number of vitally stained histiocytes that are present (Fig. 1). In normal subcutaneous tissue these cells are present only in moderate numbers, and are scattered rather widely among the more numerous fibroblasts, but in the infected tissues the ratio between ordinary fixed tissue cells and histiocytes is reversed, there being relatively more of the latter. Even at some distance from the site of the lesion this increase in the number of the vitally stained cells is quite evident. The purpose of this mobilization of the histiocytes is evident when one studies the transformation which occurs in these cells as one approaches the focus of infection; *i.e.*, the leprous nodule.

At some distance from the nodule the histiocytes show their typical morphology. They possess a well developed oval nucleus and a moderate amount of protoplasm which, on account of the mobility of these cells, is of irregular shape and which is crowded with blue dye granules. As one approaches the focus of infection one finds scattered among the normal histiocytes an occasional cell which contains fewer dye granules and whose protoplasm is swollen. Still nearer to the nodule, cells are found which contain only an occasional granule of blue and in their distended protoplasm vacuoles are present. In the immediate periphery of the mass of typical lepra cells the majority of the histiocytes have but one or two dye granules, while the greater part of their swollen protoplasmic body has all the characteristics of the typical lepra cells. There is, in other words, a gradual transformation of the histiocyte into the lepra cell.

In sections stained with carbolfuchsin one finds the bacilli in the vitally stained histiocytes (Fig. 2). There is a rough inverse variation between the number of dye granules and the number of bacilli in the protoplasmic bodies of the cells. If the cell contains many bacilli there are few granules, and those cells which contain their normal

amount of dye show only an occasional bacillus. At some distance from the nodule one finds the histiocytes therefore free of bacilli and presenting their usual deeply stained appearances. As one approaches the leprous nodule the number of phagocytosed bacilli increases, the protoplasm of the cells swells and becomes vacuolar as previously described, and the dye granules gradually disappear until, in the completely formed lepra cell of the nodule, the protoplasm contains a mass of bacilli only and no dye (Fig. 2). The other types of cells which lie around the lepra nodules and which, with proliferating capillaries, form a granulation tissue in which the histiocytes lie, take little or no part in the formation of the lepra cells. These cells are fibroblasts and round cells such as lymphocytes and plasma cells. The fibroblasts, easily identified by their morphology and lack of dye granules, though they may proliferate and form more or less collagen between and around the nodules, do not undergo any changes which could produce the swollen vacuolar lepra cells. Occasionally one sees such a cell containing a bacillus, but they remain passive and do not react to its presence. Nor do the lymphocytes and plasma cells either contain the bacilli in great numbers or are they transformed to the very different type of cell represented by the lepra cells.

In short, though the leprous nodule may contain fibroblasts and round cells, its typical cellular component, the lepra cells, is derived from the histiocyte alone. In other organs, such as the liver and lung, a similar process may be observed. In the liver it is the vitally stained von Kupffer cells which proliferate and collect around the periphery of the nodule, phagocytose the bacilli, and ultimately lose their dye granules and become typical lepra cells. In the lungs the nodules are found in the interstitial tissue, especially around the bronchi and blood vessels. Here vitally stained histiocytes are found in increased number and the same transformation of the heavily stained bacillus-free histiocyte to the bacillus-laden dye-free swollen vacuolar lepra cell is seen.

DISCUSSION.

The lepra cells when studied by means of vital staining are found to be derived from the histiocyte of the infected tissue. Fibroblasts and lymphocytes play an insignificant part in their formation although

they may be present as such in the nodule or in the granulation tissue which forms about the bacilli. If not stained vitally the histiocyte is, on account of its varying size and shape, an exceedingly difficult cell to identify and it is not surprising that these cells were confused by investigators who worked without the aid of this method, with either altered lymphocytes (Neisser;[2] Marchoux[3]) or with modified fibroblasts (Baumgarten;[4] Philippson[5]).

The origin of the lepra cell is therefore similar to the origin of the analogous epithelioid cells in tuberculosis. But there is this difference, that the lepra cell is a much better defined group as far as its origin is concerned than the epithelioid cell. The latter in the early stages of the formation of the tubercle is derived from the histiocytes, at least in certain tissues where the vitally stained cells are normally found in considerable numbers; for example, in the liver (Oppenheimer[6]), in the spleen (Goldmann[7]), in lymph glands (Joest and Emshoff[11]), and in the bone marrow (Oliver[12]). But in other tissues, such as alveolar connective tissue, where there are only moderate numbers of histiocytes, it seems certain that there is an added source of epithelioid cells in the fibroblasts. Recent work with methods of tissue culture is also indicating the possibility of their derivation from cells of the circulating blood (Lewis;[13] Maximow[14]). In other words, whereas the epithelioid cells of tuberculosis seem to be a heterogenous group derived from several sources, the lepra cells comprise a much better defined class of cell with a single origin.

CONCLUSION.

1. By means of the method of vital staining it is found that the lepra cell in rat leprosy is derived from the histiocyte.

2. From the similarity in morphology and function of the lepra cells in this condition and those of human leprosy, it seems likely that derivation of the cells in the two conditions is similar.

[11] Joest, E., and Emshoff, E., *Virchows Arch. path. Anat.*, 1912, ccx, 188.
[12] Oliver, J., *J. Exp. Med.*, 1920, xxxii, 153.
[13] Lewis, M. R., *Am. J. Path.*, 1925, i, 91.
[14] Maximow, A., *J. Infect. Dis.*, 1924, xxxiv, 549.

EXPLANATION OF PLATE 4.

FIG. 1. Vitally stained subcutaneous tissues of a leprous rat counterstained with carmine. At the left and bottom of the field are seen nodules composed of light staining lepra cells. Clustering around them and scattered throughout the surrounding granulation tissue are great numbers of dark vitally stained histiocytes. Bausch and Lomb, oc. 1, obj. 1/6.

FIG. 2. Oil immersion field showing the small nodule which lies near the blood vessel in the center of Fig. 1. Section stained with carbolfuchsin and decolorized in acid alcohol. No counterstain was used so that the only stained elements are the bacilli and the granules of vital dye, the nuclei of all cells remaining unstained. The nodule is composed of lepra cells crowded with acid-fast bacilli. At its periphery are seen the vitally stained histiocytes. Some are filled with dye granules, while others contain bacilli and show a marked decrease in dye granules. The transition between vitally stained histiocyte and the bacillus-containing lepra cell is easily seen. Bausch and Lomb, oc. 1, obj. 1/12.

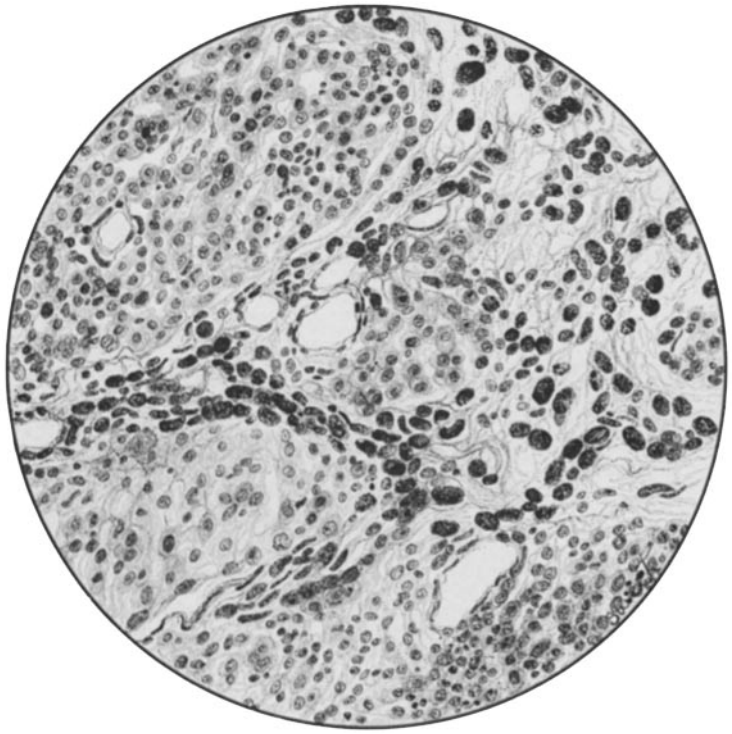

FIG. 1.

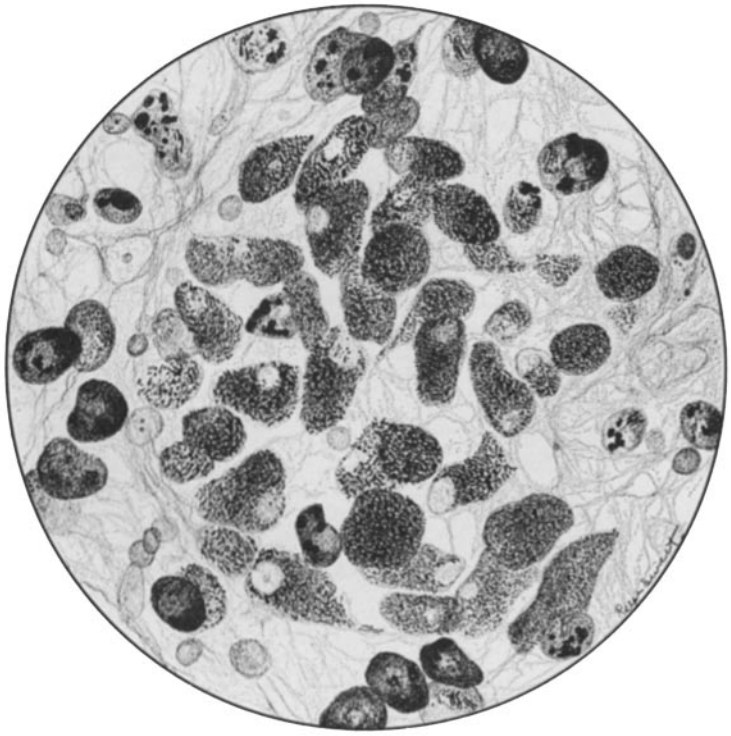

FIG. 2.

(Oliver: Origin of lepra cell.)