

THE EFFECT OF ELECTRONEGATIVE COLLOIDS ON
THE DEVELOPMENT OF RAT LEPROSY¹

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The lepra cells of rat leprosy, because of their morphologic characteristics, have been considered to be endothelial elements since the first description of the disease by Stefansky (17). This concept was subsequently studied by Marchoux and Sorel (9) and other authors, and elaborated by means of experiments with electronegative colloids, which are known to be capable of blocking the cells of the reticuloendothelial system (granuloplectic function).

Oliver (12) and Henderson (6), using trypan blue, showed that the cells which phagocytose the bacilli of rat leprosy are identical with those which retain the stain particles. Afanador (1), Lowe (8), Pinkerton and Sellards (13) and Linhares (7) confirmed these findings, the last-named worker also demonstrating an inverse relationship between the number of bacilli and the number of dye particles retained in the lepra cells.

In previous work on the development of rat leprosy (4), we have drawn attention to the fact that in this disease the lepra cells are produced mainly in organs which are rich in reticuloendothelial tissue and follow the distribution of that system. When bacilli are found in cells not of histiocytic nature, their number is always so small that the typical lepra cell is not formed.

Since there is a close relationship between the pathogenesis of rat leprosy and the reticuloendothelial system, we have thought it of interest to study the evolution of this disease in rats in which that system has been either blocked or stimulated by the action of different doses of electronegative colloids (Pittaluga (14)).

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MATERIALS AND TECHNIQUE

One hundred and fifty McCollum and Wistar rats, from 40 to 60 days old and weighing from 60 to 80 gm., were used in this experiment. They were divided into five groups of 30 animals each.

Group A: These animals were injected with trypan blue (Trypan Blau, Grübler) by the method of McClung (10), using a freshly-prepared 1 per cent aqueous suspension. Doses of 1 ml. were injected intraperitoneally each day for the first 6 days, and thereafter every third day until the end of the experiment. In this dosage the dye has a blocking effect on the reticuloendothelial system.

Group B: Using the same technique, these animals were injected with trypan blue in a 0.02 per cent aqueous suspension. Doses of 1 ml. were injected intraperitoneally on alternate days for the first 8 days, and thereafter every fifth day until the end of the experiment. This dosage was employed with the object of stimulating (exciting) the reticuloendothelial system.

Group C: These animals were injected in the same way with a colloidal suspension of carbon black (Higgin's India ink diluted with an equal volume of distilled water). Doses of 1 ml. were injected intraperitoneally on each of six successive days, and thereafter every third day to the end of the experiment. This dosage has a blocking action.

Group D: These animals were injected with Higgin's ink diluted 1:50 with distilled water in a dosage designed to stimulate the reticuloendothelial system. One ml. was injected intraperitoneally on alternate days for four doses, and subsequently every fifth day.

Group E: These animals were left untreated as controls.

Eight days after the first injections of the colloidal suspensions, the animals of all five groups were inoculated intraperitoneally with 0.5 ml. of a suspension of *Mycobacterium leprae murium* prepared as described by Hadler and Mauri (4). One animal of each group was killed on each of the first ten days after inoculation; thereafter one was killed every fifth day up to the thirtieth day, and then every tenth day until the end of the period of observation (six months).

For the purpose of this experiment, the bacteriological and histological appearances of the animals which had been "blockaded" (Groups A and C) were compared with those which had been "stimulated" (Groups B and D), and both of them with the controls (Group E). Sections of the tissue specimens were stained by the Ziehl-Neelsen method as modified by Faraco (3), or by hematoxylin and eosin, or by simple contrast staining of the background with lithium carmine.

RESULTS

Groups A and C, ("blockaded" animals).—The reticuloendothelial blockage was found usually to delay the development of rat leprosy during the first three months, the extent of the delay being directly proportional to the intensity of the blockade. Comparison of the animals of Groups A and C with those of the control group shows that the effect of the blockade is evi-

dent within a few days after the inoculation. In the controls a large number of the bacilli injected into the peritoneal cavity reach the lymphatic vessels and are retained in the retroperitoneal and mediastinal lymph nodes. Mobilization and proliferation of the endothelial cells of the marginal sinus, which phagocytose the bacilli, can be seen on the first day after the inoculation. The proliferation, mobilization and phagocytosis are more marked on the third or fourth day after inoculation, when the first leprous infiltration of the lymph nodes appears. By the fourth or fifth day the radial sinuses are reached. Up to the sixth or seventh day the primary leprous nodes are limited to the areas reached by the inoculum.

In those animals which have received large doses of electronegative colloids, sufficient to produce functional inhibition of the reticuloendothelial system, the bacilli of the inoculum are retained with difficulty in the regional lymph nodes, since the cells of the marginal and radial sinuses are already loaded with dye or carbon particles and thus are capable of only slight phagocytic activity. The appearances are distinct from those in the controls because of the smaller numbers of bacilli in the endothelial cells. The reticuloendothelial proliferation in the blockaded animals may be comparable in intensity with that of the controls, but it is more diffuse; and that is related chiefly to the effects of the colloid particles. The leprous infiltrations appear later, 5 to 7 days after inoculation. The primary nodes are formed 10 days after inoculation, and they are more circumscribed and contain fewer bacilli than are found in the controls.

The blockade hinders the development of the disease, delaying the formation and evolution of the tissue lesions during the first three months. This effect is most evident between the 45th and the 75th days; during this period the visible lesions in the blockaded animals are fewer, less extensive, noncoalescent, and poorer in bacilli as compared with the controls (see illustrations). In contrast with what occurs in the controls, the numbers of bacilli in the lesions of these animals increase slowly during the course of the disease. A similar behavior is seen in other organs in which the lesions of rat leprosy are frequent.

The delaying action becomes less marked after the third month of evolution, and is almost undetectable by the fifth month. From then on the lesions of the blockaded animals resemble those of the controls.

In short, the administration of large doses of electronegative

colloids delays the development of rat leprosy but does not arrest it. That is, those animals which have been subjected to functional inhibition or blockade of the reticuloendothelial system, when inoculated with *M. leprae murium*, show a slow development of the lesions and delayed dissemination of the disease. The same results are obtained whether trypan blue or carbon black is employed.

Groups B and D, ("stimulated" animals).—The use of small doses of electronegative colloids, which induce functional stimulation of the reticuloendothelial system, produces the opposite effect of accelerating the evolution of rat leprosy. The development of the lesions in the regional lymph nodes is more rapid than in the controls from the first day after inoculation. Those bacilli which reach the regional lymph nodes are easily phagocytosed by the endothelial cells of the marginal and radial sinuses, which are proliferated and have unimpaired functional activity. The proliferation of the reticuloendothelial system cells, in normal phagocytic activity, promotes the formation of early leprosy infiltrations (second and third days), which are more numerous and more active than in the controls. The invasion of the lymph nodes is very rapid, so that within a short time these organs are almost entirely infiltrated by the lesions. From the beginning, the lesions histologically are more diffuse and less circumscribed than in the controls.

In other organs which are rich in reticuloendothelial tissue—e.g., the liver, spleen and bone-marrow—it was observed that the functional stimulation invariably acted in the same way. In the animals of these groups, tissue specimens taken 60 days after inoculation showed exuberant lesions, resembling in extent and in the numbers of bacilli those taken from control animals 120 days after inoculation (see illustrations).

After the third month the accelerated development of the lesions becomes less evident, and at about the fifth month it is practically undetectable.

In short, in animals subjected to reticuloendothelial stimulation the lesions showed more rapid development and greater numbers of bacilli than in the controls.

DISCUSSION

The repeated injection of electronegative colloids results in the production of increased numbers of reticuloendothelial cells, not only by division of the histiocytes in granuloplectic function, but also by stimulation of the fixed cells. The proliferation of

histiocytes may, in some cases, of itself give rise to nodules of granulomatous appearance. Granulopexy and proliferation are concomitant phenomena.

When concentrated suspensions are used, the retention of the colloid particles is greater than the proliferation, resulting in blocking and functional inhibition. On the other hand, when small doses of dilute suspensions are used, the effects of reticuloendothelial proliferation outweigh those of the retained particles, resulting in functional stimulation.

Because the reticuloendothelial system plays an important role in the defence mechanism, chiefly by its phagocytic activity, it is obvious that the administration of massive doses of an electronegative colloid will influence the bodily resistance to infection by disturbing the phagocytic function. In general, whenever the reticuloendothelial system of an animal is blocked in this way, inoculation of a pathogenic agent results in more rapid and more severe development of the disease.

The results of the present study indicate that this does not hold good in the case of rat leprosy. As stated, the injection of concentrated suspensions of electronegative colloids delays considerably the development of the disease; the leprosy lesions are smaller and less numerous, develop more slowly, and contain fewer bacilli than those of controls. On the other hand, the injection of dilute suspensions promotes the development of the infection, with the formation of large nodules in many organs and more rapid progress of the disease than in control animals. These findings, which are in opposition with the usual observations in bacterial infections, are related to the peculiar pathogenic and biological characteristics of *M. leprae murium*.

It is generally found that reticuloendothelial cells which are already loaded with electronegative colloid particles can only with difficulty take up particles of another substance, even when the latter is present in the blood in high concentration. When retention does occur, the new colloid is deposited around the granules of the first one.

On the other hand, in both granulopexy (or athrocytosis) and in phagocytosis, the first phase—that of adsorption on the cell surface—is essentially similar. There is some difference of opinion concerning the subsequent phases. Some workers, as Möllendorf (11), regard them as representing two different phenomena, while others as De Haan (2) and Seemann (15), consider them to be alike. Nevertheless, it is generally held

that there is a certain resemblance between athrocytosis and phagocytosis, which may be regarded as correlated though not identical phenomena.

In rat leprosy there is clear evidence of the influence of granuloplectic activity upon phagocytosis of the bacilli. Cytological studies show that histiocytic cells which are loaded with colloid particles have a reduced capacity to phagocytose the bacilli. The effect of cell blockade is clearly seen in histological sections, when an inverse relationship can be observed between the number of bacilli and the number of retained particles, as was stated by Linhares (7). It has been shown by Oliver (12), Henderson (6), and Linhares (7) that the large lepra cell, in which the cytoplasm is loaded with bacilli and the nucleus is frequently pycnotic and displaced peripherally, has a limited capacity to take up the particles of electronegative colloids.

Our findings show that in animals subjected to reticuloendothelial blockade, the Stefansky bacilli succeed in penetrating the cells and multiplying in them. Proportionately as the bacilli increase in numbers, the granuloplectic activity of these cells diminishes progressively, in spite of repeated injections of the colloid suspension. In consequence, the influence of the colloidal particles is progressively smaller, while the leprotic infection increases. Since the inhibitory action on the evolution of the lesions is effective only over a limited period, the use of high doses of concentrated colloidal suspensions has no practical therapeutic application.

A study of these facts shows: (1) that the retention of large numbers of colloid particles by the reticuloendothelial cells, which diminishes their granuloplectic and phagocytic activities, has an inhibitory effect on the development of rat leprosy, an effect related to the "antixenic inhibition" of the reticuloendothelial system; and (2) that the retention of smaller numbers of colloid particles induces a functional stimulation of the histiocytic system with cell proliferation, each cell still having normal phagocytic and granuloplectic activities. Under such conditions the disease progresses rapidly.

These observations are in agreement with those of Hanks (5), who has reported a favorable effect of injections of carbon suspensions on the development of subcutaneous lepromata in the early stages of the infection.

It will be seen that in rat leprosy, unlike other diseases, phagocytosis of the bacilli offers no defence against the infection, but is actually necessary for the normal development of

the disease. These facts can be explained only if one accepts the view that the Stefansky bacillus is normally capable of reproducing only after being phagocytosed by the histiocytic cells—i.e., that the bacillus requires a cellular cytoplasm for normal reproduction, as in human leprosy (16). Both the reticuloendothelial blockade, which causes a reduction of the phagocytic activity, and the stimulation of that reticular system, which causes an increase of this function, would thus have an indirect influence on the multiplication of the bacilli.

The bacteriemia is similar in intensity and duration in both those rats subjected to reticuloendothelial blockade and control animals. The reduced phagocytic capacity of the histiocytic system of blocked animals would be expected to result in an increased bacteriemia, if the rat leprosy bacillus had a normal extracellular reproduction. Therefore the available evidence suggests that, in rat leprosy, parasitism is limited to the cells of the reticuloendothelial system. Furthermore, by decreasing the phagocytic function, the blocking of this system would indirectly inhibit the reproduction of the bacilli. Nevertheless, we do not wish to dismiss altogether the possibility of extracellular reproduction, which however would have a secondary role.

By decreasing both the phagocytic activity of the histiocytes and the capability of the bacilli to reproduce, reticuloendothelial blockade has a delaying effect on the evolution of rat leprosy. In contrast, stimulation of the histiocytic system, by promoting cellular proliferation as well as increasing phagocytic ability, assists the bacilli to multiply and consequently accelerates the development of the disease.

SUMMARY

The authors have studied the development of rat leprosy in rats which, prior to inoculation with *M. leprae murium*, were injected with electronegative colloidal suspensions, trypan blue or carbon black, at varying dosage levels with the object of producing "blocking" of the reticuloendothelial system by high dosage or "functional stimulation" by low dosage. The results observed in animals receiving blockading doses is compared with those in animals receiving stimulating doses, and with those in controls.

It has been found that high doses, which cause functional inhibition of the reticuloendothelial system, delay the evolution of rat leprosy but do not prevent it; the development of lesions is slowed and the spread of the infection is delayed.

On the other hand, in those animals subjected to reticuloendothelial stimulation, the lesions appear earlier, are more extensive, develop more rapidly and contain significantly larger numbers of bacilli than in control animals.

These findings may be explained on the basis of the correlation existing between phagocytosis and granulopexy, which exerts an influence on the intracellular reproduction of the bacilli.

By reason of the reduction of phagocytic activity, which diminishes the reproductive capacity of the bacillus, reticuloendothelial blockade delays the development of the disease. In contrast, reticuloendothelial stimulation, by causing increased cellular proliferation and hence greater phagocytic activity, facilitates reproduction of the bacilli and, in consequence, the development of the disease.

In rat leprosy, unlike other diseases, phagocytosis of the bacilli offers no defence against the infection; it is actually necessary for the development of the disease. These facts can be explained only if one accepts the view that the Stefansky bacillus is normally capable of reproduction only after phagocytosis by the histiocytic cells, i.e., that the bacillus requires a cellular cytoplasm for normal reproduction, as in the human leprosy.

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DESCRIPTION OF PLATES

All sections stained by hematoxylin-eosin. All photomicrographs made at 200 × magnification.

PLATE 12.

FIG. 1. Lymph node, rat 251; blockade of the reticuloendothelial system. Cortical nodule constituted by histiocytic cells containing few bacilli. Note areas in the medullary zone showing intense granulopexy.

FIG. 2. Lymph node, rat 202; stimulation of the reticuloendothelial system. Numerous nodules constituted by histiocytic cells rich in bacilli.

FIG. 3. Lymph node, rat 327; control. Leprosy nodules in the normal evolution of the disease on the 30th day.

(NOTE: Figs. 1-3 are of material taken on the 30th day after inoculation.)

FIG. 4. Lymph node, rat 288; blockaded reticuloendothelial system. Little nodules the cells of which show intense granulopexy; some bacilli in the lesions.

FIG. 5. Lymph node, rat 232; stimulated reticuloendothelial system. Numerous and coalescent nodules; lesions very rich in bacilli.

FIG. 6. Lymph node, rat 144; control. General aspect of the lesions in the normal evolution of the disease on the 70th day.

(NOTE: Figs. 4-6 are of material taken on the 70th day after inoculation.)

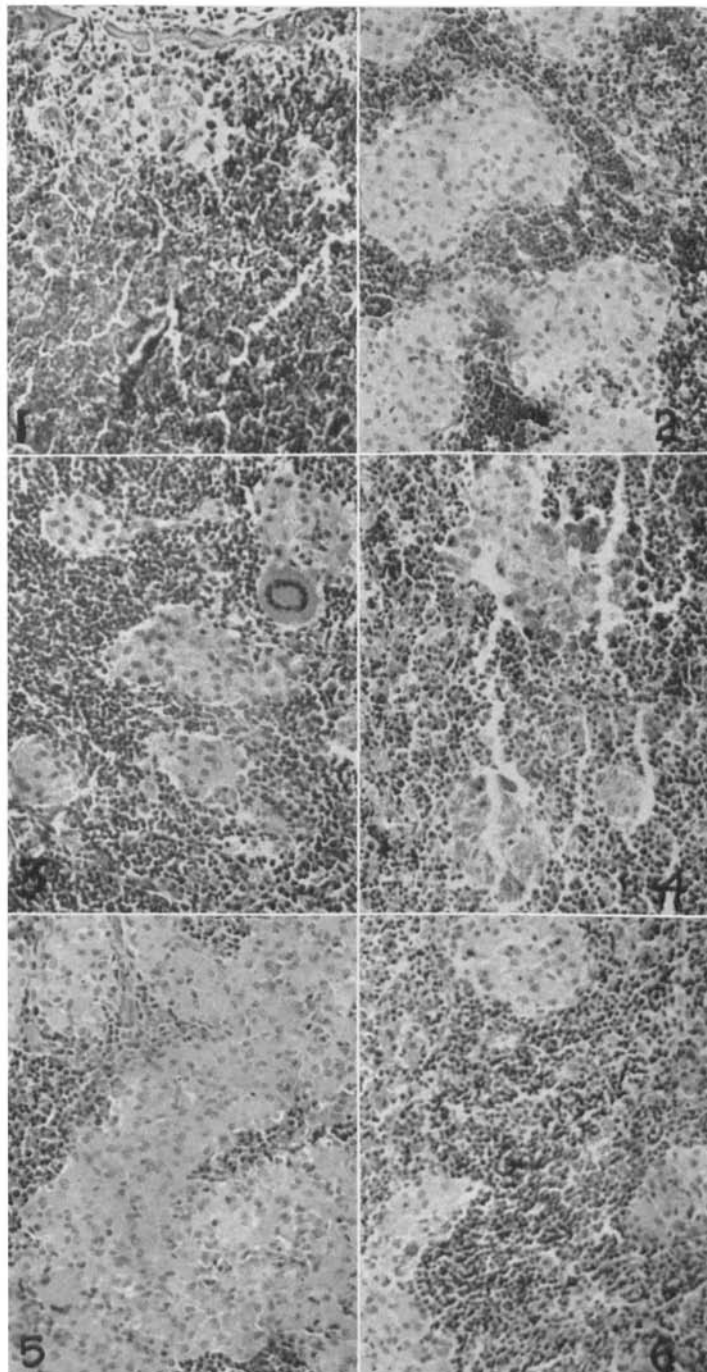


PLATE 12.

PLATE 13

FIG. 7. Lymph node, rat 193; blockaded reticuloendothelial system. Showing a few small, poorly delimited nodules.

FIG. 8. Lymph node, rat 261; stimulated reticuloendothelial system. Coalescent leprotic nodules, with marked reduction of the lymphoid tissue.

FIG. 9. Lymph node, rat 310; control. Aspect of the leprotic nodules in the normal evolution of the disease on the 100th day.

(NOTE: Figs. 7-9 are of material taken on the 100th day after inoculation.)

FIG. 10. Lymph node, rat 224, blockaded reticuloendothelial system. Proliferation and mobilization of the histiocytic cells in granuloplectic activity, grouped in a way which simulates incipient leprosy lesions but without bacilli; 70 days after inoculation.

FIG. 11. Liver, rat 288, blockaded reticuloendothelial system. Small perivascular leprosy nodules constituted by histiocytic cells containing a few bacilli and a large quantity of granules, 70 days after inoculation.

FIG. 12. Liver, rat 168, control. Perivascular leprosy nodule in the normal evolution of the disease, 70th day.

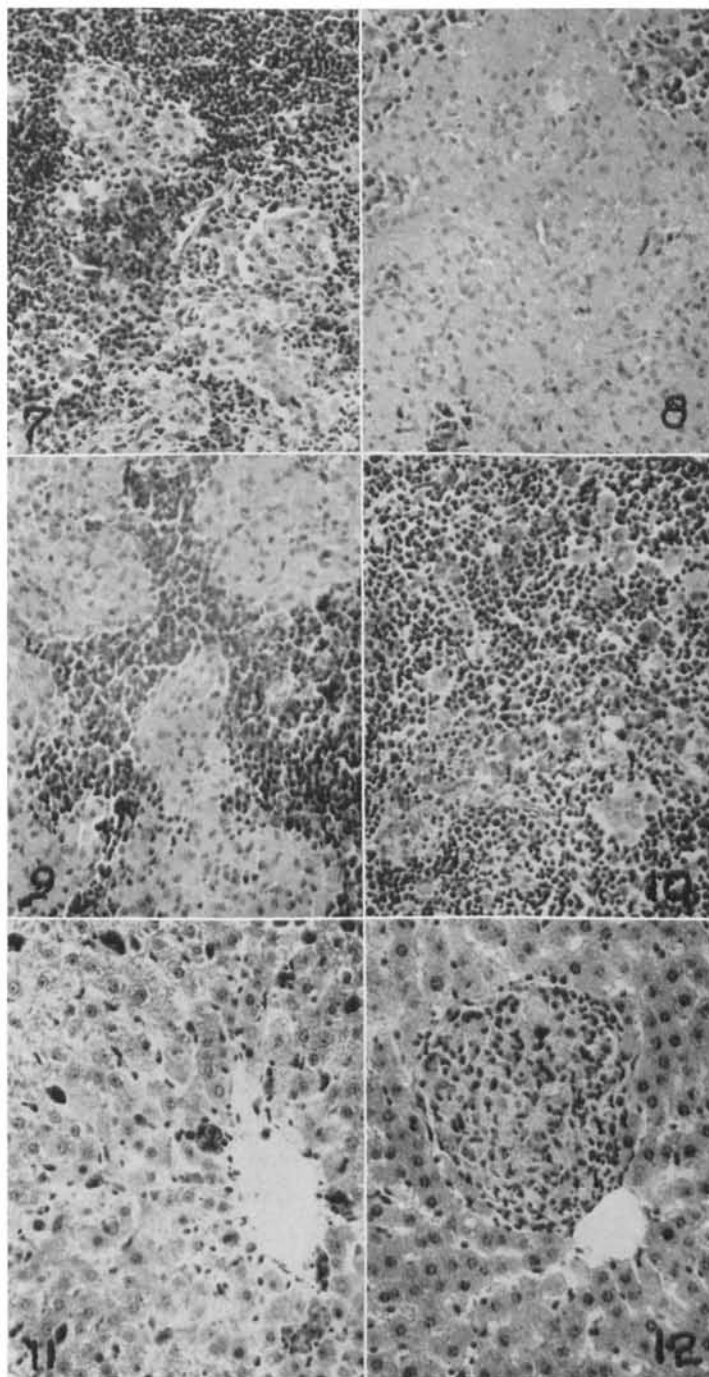


PLATE 13.