THE PATHOLOGY OF EXPERIMENTAL DERMAL PNEUMOCOCCUS INFECTION IN THE RABBIT

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Plates 2 and 3

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The development and progression of the morphological changes in the human pneumonic lung are still incompletely understood, a fact partly explainable by the circumstance that pneumonic lesions, lobar in distribution, can be produced in animals only with difficulty. In previous papers (1, 2) one of us has described an experimental disease brought about by infecting rabbits intradermally with pneumococci (Type I). Although in this infection the focus is in the skin, there exist certain clinical and immunological similarities with lobar pneumonia in man; moreover, the visible location of the principal lesion is a definite aid to experimental studies. Observations made in the course of other experiments of this nature suggested that a study of the morphological alterations, both gross and microscopical, might lead to a better understanding of the fundamental phenomena of pneumococcic infection irrespective of the site of the lesion.

It may be recalled from an earlier report (1) that if a normal rabbit is given an intradermal inoculation of virulent pneumococci on the flank a local infection develops in the course of several hours. The lesion soon begins to spread ventrally and within 24 hours occupies a large area extending from the site of inoculation across the ventral midline. The infection is characterized particularly by the production of massive edema.

The course of the development of the lesion, as previously reported (1), may be summarized as follows:

After the intradermal injection of 0.2 cc. of an 18 hour blood broth culture there is at first a latent period of some 2 hours. Near the site of inoculation there then appear signs of early inflammation; these are characterized at first by conges-

tion of vessels and capillaries; the sharp vascular outlines are then lost and an area 1 to 2 cm. in diameter becomes slightly edematous and shows a trace of orange-red color. As more edema develops the accumulation of fluid is most pronounced at the ventral border. The lesion spreads ventrally at a rate of 2 to 3 cm. an hour, the heightened color being apparently preceded by the movement of fluid. At 10 to 12 hours the edematous band has usually extended to the ventral midline. More and more serous material accumulates in this region until the entire area is tense and swollen. Within 18 to 24 hours a certain degree of induration is present and this increases gradually until at 48 hours the involved tissue is firm and relatively non-elastic.

At 24 hours the lesion usually occupies a strip 2 to 3 cm. wide on the flank increasing to a width of 4 to 6 cm. at the ventral midline. Beginning at 24 to 30 hours there is a marked contraction of the involved tissue, and large irregular folds develop over the more ventral section. The color of the lesion varies from a pale to a moderately bright orange-red. In the infections which appear to be the more severe and fatal the involved skin often takes on a hemorrhagic character frequently evidenced by scattered ecchymoses or by widespread hemorrhage. Some frank necrosis with secondary infection may take place, the incidence varying with the amount of trauma to which the tissue has been subjected.

Methods

In order to study the histological development of this dermal lesion, a series of normal animals were injected intradermally with 0.2 cc. of an 18 hour blood broth culture of Pneumococcus Type I. The site of injection in each instance was at a point on the animal's flank approximately 8 cm. dorsal to the ventral midline. The development of the lesions and the concomitant temperature reactions were then carefully followed.

Except in one instance the animals were killed at various intervals after infection by inducing air embolism. The entire skin lesion, including the underlying muscle and peritoneum, was removed, lashed to cardboard to prevent distortion and by a longitudinal incision divided into two equal pieces. One was fixed in Zenker-formol and the other in Zenker's fluid to which 5 per cent of glacial acetic acid had been added. Multiple blocks, suitable for study of the entire extent of the lesion were embedded in paraffin, sectioned, and stained with hematoxylin-eosin, eosin-methylene blue, and Mallory's phosphotungstic acid hematoxylin. Stains for bacteria were made according to the method of Brown (3).

HISTOLOGICAL OBSERVATIONS

The earliest lesion to be examined was removed 2 hours after infective inoculation and before any elevation of the animal's temperature had taken place.

The only external evidence of disease was a soft, pale area of very slight swelling about the point of inoculation. Histological examination shows a marked accumu-

lation of edema fluid in the corium and in the loose adipose and connective tissue between corium and muscle. A moderate number of polymorphonuclear leukocytes are present although fibrin is not seen nor are erythrocytes in evidence in any considerable number. The muscle layer and corium show scattered infiltration with leukocytes. Blood vessels, particularly the smaller ones, are congested, dilated, and appear more numerous than in the normal skin. The lymphatics also appear larger and more numerous but contain few leukocytes and no fibrin. Study of the borders of the lesion indicates clearly that extension of the edema fluid takes place in advance of infiltration with inflammatory cells. No evidence of tissue necrosis can be made out. Nearer the center of the lesion, presumably in relation to the site of inoculation, a marked accumulation of mononuclear wandering cells has taken place in and around the adventitia of the blood vessels. A photograph of the lesion at this stage is reproduced in Fig. 1. This shows the early histological development with edema distending the corium and subcorial space. Fig. 2 shows the uninvolved tissue just peripheral to the lesion.

The progression of the histological alterations was next studied in a lesion taken at 5 hours.

This animal's temperature had risen to 104°F., an elevation of a little over a degree from the normal. The involvement at this time measured 2.5 by 3.5 cm. the latter being the dorsoventrad dimension. This area was definitely edematous, a feature most prominent at the ventral border. The color except for the most ventral portion was a faint orange-red and the consistency very soft.

At this time the histological picture generally parallels that described at 2 hours, but all features are more marked. The loose connective tissue fibers of the corium and panniculus adiposus have been widely separated from each other by the edema fluid, and this has resulted in a structure which, on superficial examination, appears not unlike the areolar structure of pulmonary parenchyma. The relatively thin tissue layer has thus been converted into the outstanding anatomical structure. The cellular infiltration has extended into the deeper layers of the corium but as yet is not a marked feature. The dilatation of the lymphatics and the increase in size and number of functioning vessels present a picture similar to that seen in the earlier specimens. Despite the size and rapid progression of the lesion, the response in the form of cellular exudate is only moderate in degree. Necrosis of tissue cannot be detected, nor can any deposition of fibrin be observed. Although dilated lymphatics and engorged blood vessels form a prominent feature at this stage, even more so than in the earlier lesion, no evidence of thrombosis can be found.

The next tissue studied was removed from an animal 8 hours after infective inoculation.

The rabbit's temperature had risen to 105°F. The lesion occupied an area 2.5 by 8.5 cm. extending to and just beyond the ventral midline. The entire zone was soft and edematous, a change most pronounced at the more ventral projection. Near the point of original inoculation the color was a very bright orange-red but this shaded off gradually until the ventral third of the area was of the same color as the surrounding tissue. At this time sections show that there had been a distinct shift in the predominant cell type of the inflammatory reaction. The perivascular grouping of mononuclear phagocytic cells is a striking feature, many cells of this type having also taken part in the general cellular response. Edema is still the main feature of the lesion and has completely separated corium from muscle. Infiltration, both of polymorphonuclear and mononuclear cells, is steadily increasing in degree and extends, at this time, well into both corium and muscle layers. Still no hemorrhage or necrosis is present. The degree of lymphatic dilatation does not appear to be altered but more small blood vessels, greatly engorged, are appearing in the corium.

The further progress of the pathological processes was studied in a lesion removed at 28 hours.

The animal's temperature had been over 104°F. for approximately 20 hours, with a maximum high level of 105.5°F. The lesion at this time occupied a strip 3 cm. wide extending downward from the point of inoculation to the ventral midline where the involvement measured 8 cm. in diameter. The entire area was greatly swollen, an alteration accentuated by the formation of huge folds caused by the contraction of the skin. The lesion was rather firm, especially in the area near the site of inoculation. The color varied from a pale to a bright orange-red. No macroscopic necrosis or hemorrhage was apparent. Microscopically, the sections show at the site of maximum involvement, near the juncture of upper and middle third of the lesion, the earliest evidence of death of the cells forming the exudate. Here groups of acidophilic mononuclear and polymorphonuclear leukocytes are seen. Peripheral to this reaction, the picture previously described of corial and subcorial edema associated with mild inflammatory infiltration is encountered. Perivascular cell infiltration is still marked and the large number of newly formed or newly functioning capillaries is most striking. The lower edge of the lesion, on the other hand, as the most recently involved, presents changes not unlike those seen in the preparations previously described. Here, the edema, both in the subcorial space and in the inferior layers of the corium, is by far the most prominent change. Inflammatory exudate is sparse and diffuse.

As an example of the fully developed lesion, tissue was studied 2½ days after infection.

This animal died under observation after a typical course of the disease associated with high temperature. The tissue was excised and fixed immediately

after death. The lesion occupied an area of much the same size as that last described. The edema was massive, firm, and circumscribed. A bright orange-red color, most pronounced in the early phases, was succeeded at 45 hours by ecchymoses which rapidly progressed to hemorrhagic necrosis. At this time a radical alteration appears in the histological picture at the oldest part of the lesion, situated well toward the dorsal end. Here, in addition to the edema previously described, fairly dense infiltration composed of mononuclear and polymorphonuclear leukocytes is found in the lower layers of corium and the strata of the upper muscle layers. Groups of necrotic inflammatory cells are occasionally encountered although necrosis of parenchyma is not apparent. Diffuse hemorrhagic infiltration is striking in certain areas. Peripheral to this well established lesion, there are regions presenting rather less activity, where the edema is most prominent and the cellular infiltration not marked. A large number of new or newly functioning capillaries and even larger vessels have appeared, many surrounded by a collar of mononuclear cells. Some proliferation of fibroblasts has taken place in the lower corium. The epithelial surface presents a number of small areas where the cells have undergone a degenerative change overlying a base of coagulated exudate containing roughly parallel rows of polymorphonuclear and mononuclear leukocytes. Subepithelial lymphatics are greatly distended. Inflammatory infiltration extends well up to the epithelium and down to the second layer of muscle. A certain number of muscle fibers appear necrotic. Thrombosis of both large and small veins is well marked in this preparation, a condition presumably accountable for the marked hemorrhage present.

The last example studied was that of an advanced lesion, removed at 99 hours after infection.

This presented essentially the same gross pathological picture as that just described, except that the hemorrhagic condition was never so prominent. Microscopically, this lesion shows a picture unlike that seen at any other period studied. The process is one of repair and of reaction to foreign material represented by groups of cellular débris. Much edema is still present and there is a massive infiltration with leukocytes, predominantly mononuclear in type. Many new vessels and young active fibroblasts are seen, a change particularly marked around the pockets of necrotic material. Thrombosis is a marked feature. The extensive edema and scattered cellular infiltration, so marked in the earlier slides, is not apparent in this later preparation. Figs. 3 and 4 show low and high magnifications of a section of a mature lesion.

These examples show the course of development and maturation of the lesion. Studies on the histological changes occurring during resolution are now in progress and will be presented in connection with certain data bearing on the recovery process.

DISCUSSION

In order to relate these observations to the problem of the early morphological alterations in lobar pneumonia, it is first necessary to consider in some detail the changes which take place in the human lung. Briefly, these changes have been described as engorgement, red hepatization, gray hepatization, and resolution.

The first stage, that of engorgement, is not commonly seen at autopsy and the descriptions for the most part have been derived from study of tissue contiguous to a pneumonic process in the direction in which the pneumonic process is presumed to be spreading. Here engorged vessels may often be found. More commonly, however, the only evidence of progression is the accumulation, in the alveoli, of edema fluid containing a few polymorphonuclear leukocytes. Blake and Cecil (4), as well as Winternitz and his coworkers (5) have called attention to the dilated lymphatics in experimental pneumococcus pneumonias in animals. Permar (6), on the other hand, studied the lesion of experimental pneumonias sooner after infection than was done by the workers just mentioned and pointed out that the most striking change was the presence of intraalveolar fluid in the absence of lymphatic involvement. Loeschcke (7), in a detailed study of the histological alterations in the human pneumonic lung, has called attention to the prominence of edema formation in the early and advancing lesions.

Comparing these changes, considered to be the earliest manifest in the pneumonic lung, with the alterations observed in the infected skin of the rabbit a remarkable similarity is immediately evident. The accumulation of a relatively cell-free exudate and the presence of dilated lymphatics, dilated and engorged capillaries, and an intact supporting structure are all found in the dermal infection. The progression of the infection by the production of edema fluid, presumably containing the infecting bacteria, would suggest strongly that a similar mechanism may obtain in the pneumonic lung.

The stage of red hepatization is only rarely encountered in lobar pneumonia. It is usually described, however, as being characterized by a deep red color, presumably due to dilated and engorged capillaries of the alveolar wall. The exudate stains well and contains a considerable number of polymorphonuclear leukocytes and a certain amount of fibrin, although the latter is a variable factor. The interstitial tissue is still intact and the lymphatics are dilated, containing a certain number of inflammatory cells.

Turning to the lesion observed in the rabbit skin, the same general pathological alterations are found. Vascular congestion is a marked

feature and the inflamed area appears red on gross inspection. The exudate near the site of inoculation now contains a larger number of inflammatory cells, mostly polymorphonuclear leukocytes, as well as a few mononuclears. The number of erythrocytes is extremely variable. Fibrin may be present but it is not a striking feature. The lymphatics are markedly dilated and may contain a moderate number of leukocytes. As in the lung no evidence of damage to the supporting structure is encountered. At the periphery of the progressing lesion, the changes are identical with those of the initial reaction present throughout when the tissue is examined a few hours after inoculation. Exactly the same condition may be encountered in the human pneumonic lung, where, at the periphery of the consolidated tissue, areas may be found in which the alveoli contain edema fluid with a few cells and the vessels are greatly engorged, a picture presumed to be widespread early in the disease. As in the lung no necrosis is to be found, so also, the interstitial tissue of the rabbit skin is quite intact.

The third stage of lobar pneumonia, that of gray hepatization, is the one most frequently encountered at autopsy. Here, the fibrin of the exudate is commonly most dense and shows a tendency to shrink away from the alveolar walls. The cellular exudate, composed for the most part of polymorphonuclear and mononuclear leukocytes, stains poorly and is presumed to be largely necrotic. The small vessels in the walls of the alveoli are inconspicuous and may contain no blood whatever though this can hardly be stated to be the rule. A number of mononuclear phagocytic cells have appeared by this time, and many contain droplets of fat or fine granules of hemosiderin.

The comparison with the more advanced lesion of the rabbit dermal infection is a striking one. Here, corresponding to the pneumonic lung, one finds that the oldest exudate is necrotic and that mononuclear phagocytes are engaged in carrying away various broken-down products. Capillary engorgement is by no means so marked and although many new vessels have appeared they are in association with granulation tissue, a change easily understood when one considers that in the skin the exudate has no ready route of exit. The removal of the exudate must take place more slowly and a certain amount of organization is bound to occur. Necrosis of dermal tissue is, however, not a feature and in this respect the analogy with the pneumonic lung still holds. This analogy is also true in the case of the fibrin which is

much more dense in both of the lesions at this stage. One feature deserves especial attention and that is the occurrence of hemorrhage. In the dermal lesion this is fairly striking in certain of the more advanced stages but it has not been commonly described in discussion of the pathology of lobar pneumonia. Loeschcke (7) has recently called attention to the occurrence of intraalveolar hemorrhage in the later stages of lobar pneumonia. This author explains his finding by the shrinkage of the fibrin plugs away from the alveolar walls, allowing a decrease of pressure on the alveolar side of the membrane and consequent rupture of the weakened vascular wall with resultant outpouring of red blood cells into the alveoli. It is undoubtedly true that hemorrhagic areas are frequently observed in the pneumonic lung at autopsy and a detailed microscopic study of a limited amount of material available to us has revealed a surprising amount of intraalveolar hemorrhage in areas of the lung known from clinical data to represent the older pathological processes. In such preparations thrombosis of the finer vessels appears to give rise to this phenomenon and here again the same condition is found in the skin lesion.

In recapitulation, it may be said that the predominant change in the rabbit skin, infected with pneumococci, appears to be the production of an extraordinary volume of edema fluid. This fluid, carrying pneumococci, seems to spread rapidly, filling the interstices of the connective tissue framework. The direction which the fluid takes is influenced principally by gravity. Following the production of fluid there appears a cellular exudate, predominantly polymorphonuclear but in the later stages containing also many mononuclear cells. As the age of the lesion increases more fibrin is seen and eventually the oldest exudate becomes necrotic, although at the border the relatively pure edematous lesion is still present. Hemorrhage may be a late manifestation. Since changes of the same general nature may be observed in the pneumonic lung, it is of some interest to consider the likelihood that the pulmonary disease may extend by a similar mechanism, that is to say, by the production of an infectious edema fluid near the hilus with secondary spread of this fluid by force of gravity and by the churning action of the moving alveolar walls.

The chief immediate significance of these findings is that they establish the fundamental similarity of the pathological processes of the

rabbit dermal disease and those of human lobar pneumonia. This similarity has importance for work to be reported later on factors which affect the development, progression, and localization of the rabbit lesion, as well as in relation to the method that has been suggested for the comparison of the curative properties of antipneumococcic sera by use of this experimental infection (8).

SUMMARY

1. The pathology of the experimental dermal pneumococcic infection in the rabbit is described in detail and the findings are compared with the histological alterations seen in the human pneumonic lung. There would appear to be a basic similarity of the lesions in both tissues.

2. A copious production of edema fluid is the outstanding characteristic of the early lesion. It occurs prior to any significant cellular change. In the spreading lesion an infiltration of the tissues with fluid precedes any other sign of reaction between tissue and microorganism. It seems likely that the advancing fluid carries with it the infecting organisms and inoculates all tissues which it reaches. The resulting infection seems not to take place by an active invasion of microorganisms but by a progressive inoculation from an infected fluid.

BIBLIOGRAPHY

1. Goodner, K., *J. Exp. Med.*, 1928, **48**, 1.
2. Goodner, K., *J. Exp. Med.*, 1928, **48**, 413.
3. Brown, J. H., and Brenn, L., *Bull. Johns Hopkins Hosp.*, 1931, **48**, 69.
4. Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, **31**, 445.
5. Winternitz, M. C., Smith, G. H., and Robinson, E. S., *Bull. Johns Hopkins Hosp.*, 1920, **31**, 63.
6. Permar, H. H., *J. Med. Research*, 1923, **44**, 1.
7. Loeschcke, H., *Beitr. path. Anat. u. allg. Path.*, 1931, **86**, 201.
8. Goodner, K., *J. Immunol.*, 1929, **17**, 279.

EXPLANATION OF PLATES

PLATE 2

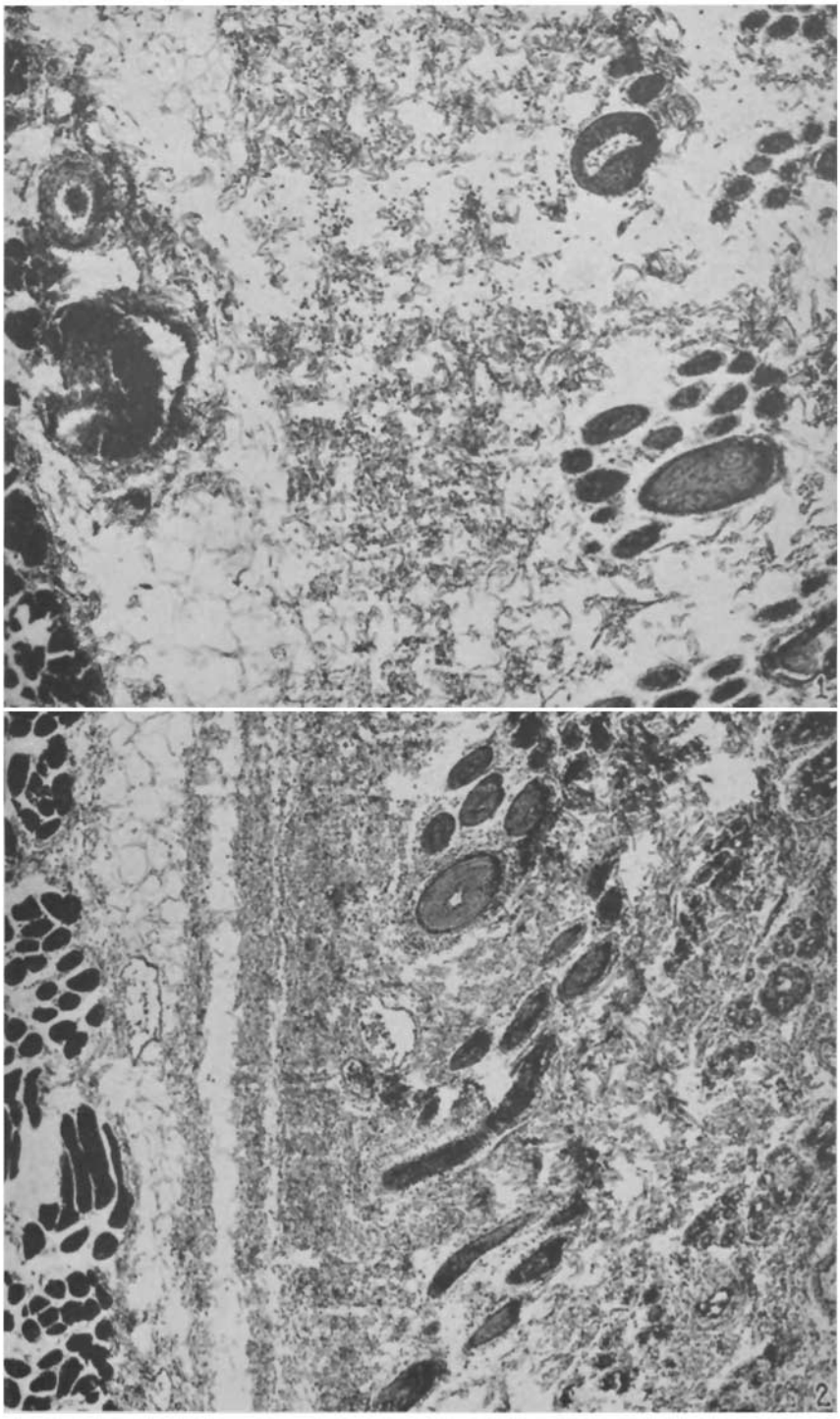
FIG. 1. Photomicrograph of a section of a lesion from the skin of a rabbit 2 hours after infection with Pneumococcus, showing marked edema of corium and subcorial space associated with slight cellular infiltration. × 65. Phosphotungstic acid hematoxylin.

FIG. 2. Same section, showing relatively unaffected tissue beyond the periphery of the lesion depicted in Fig. 1. × 65. Phosphotungstic acid hematoxylin.

PLATE 3

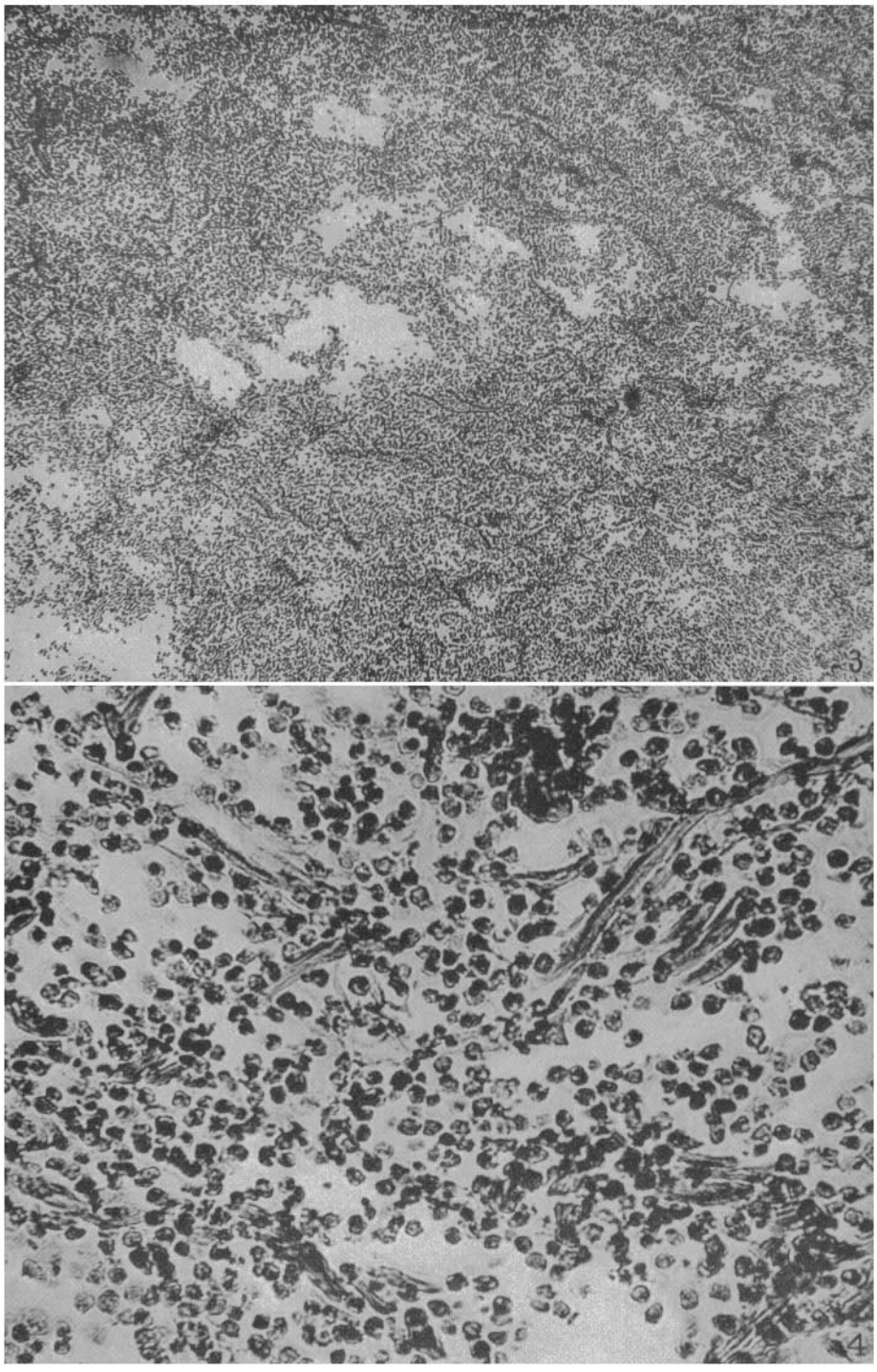
FIG. 3. Low power photomicrograph of corium showing the intense inflammatory infiltration in the mature lesion. × 65. Eosin-methylene blue.

FIG. 4. Same section, with higher magnification, indicating the marked edema, the character of the cellular infiltration, and the separation of connective tissue fibrils. × 550. Eosin-methylene blue.



Photographed by Louis Schmidt

(Rhoads and Goodner: Dermal pneumococcus infection)



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