STUDIES ON CHANCROID

I. Observations on the Histology with an Evaluation of Biopsy as a Diagnostic Procedure *

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This report is the first in a series of studies dealing with the clinical, bacteriologic, and histologic aspects of chancroidal infection. In this paper the histologic appearance of chancroid is described and biopsy as a diagnostic procedure is discussed.

There is definite need for a simple and accurate laboratory method by which the diagnosis of chancroid can be established.[1,2] This has been emphasized recently by the statement from the Surgeon General of the United States Army that the usual laboratory tests are not recommended.[3] The Ducrey skin test was thought to be diagnostic, but Knott and his associates [4] have shown that this test is of limited value. Auto-inoculation is not generally used in the diagnosis, since it produces a new infection. The identification of the Ducrey bacillus is the most accurate method of diagnosis but the demonstration of the organism in direct smears of the lesion has many pitfalls,[5] and isolation by culture is not generally attempted.

In this study cultures were taken from all lesions from which tissue had been obtained for biopsy. The identification of the Ducrey bacillus confirmed the histologic diagnosis in our cases and offered, at the same time, an opportunity to compare the usefulness of culture and biopsy. To our knowledge no similar studies have been reported in which a large group of cases were investigated concurrently by histologic and bacteriologic methods.

Method of Study

The histologic study is based upon 45 specimens taken for biopsy, which were diagnosed as chancroid. They occurred among 59 specimens taken from 125 patients who had been selected for a special study of lymphogranuloma venereum and chancroid. These patients were subjected to a number of diagnostic procedures which included skin tests, microscopic examination of stained smears, darkfield preparations, lymphogranuloma complement-fixation tests, serum protein determinations, biopsy, auto-inoculation, culture, and inoculation of mice and chick embryos.

* Aided by a grant from the Venereal Disease Division of the United States Public Health Service.

Received for publication, April 30, 1945.

Tissue specimens were taken without anesthesia with a Gaylors biopsy forceps from the base of the ulcer, usually with little pain. Care was taken to avoid the adjacent skin, and bleeding was easily controlled by local pressure. The specimens averaged 3 mm. in diameter; they were fixed immediately in Zenker's fluid containing 5 per cent glacial acetic acid. Mallory's phloxine-methylene blue stain was used routinely, but in some instances Giemsa's and Gram's stains were also used. The histologic diagnoses were made within 24 hours, and were returned before the results of the cultures were known.

Cultures were taken from the primary lesion or from the auto-inoculation in every case. A modification of the Teague and Deibert method [6] was used and the culture was reported as positive only when the typical tangled chains of gram-negative coccobacilli, the Ducrey bacillus, were found. A discussion of the technic of culture is to be reported elsewhere.

There were 28 male and 17 female Negro patients in this series. The lesions varied in duration from 2 days to 4 weeks. In two instances the lesions occurred in the upper vagina or on the cervix, while in the remainder the lesions were located on the external genitalia.

Histologic Observations

Little information on the histologic appearance of chancroid is found in the standard textbooks of pathology, although a description of some features is given by Moore.[7] He described an intra-epidermal abscess extending into the dermis, perivascular infiltration by polymorphonuclear leukocytes and a few plasma cells, dilatation of blood vessels and lymphatics, and marked acanthosis of the epithelium at the edge of the ulcer.

According to McCarthy,[8] the "most typical histologic picture" is an ulcer which is generally confined to the upper corium, but may extend into the subcutaneous tissues. There is moderate acanthosis of the epithelium. Lymphocytes and plasma cells participate to an approximately equal degree in the diffuse and dense inflammatory infiltration. Polymorphonuclear leukocytes predominate on the ulcerated surface. The inflammatory cellular infiltration blends with the adjoining tissue where it forms perivascular cuffs. The blood vessels and lymphatics are dilated and the former show marked endovasculitis and perivasculitis.

Pund, Greenblatt, and Huie [9] described similar changes and stressed the marked swelling of the endothelium which obstructs the capillaries and leads to necrosis. They considered the vascular changes as "characteristic and responsible for the superficial necrosis and unhealthy granulation." Von Haam [10] felt that these changes represent only a

superimposed fusospirochetal infection. In his observations he noted the absence of any vascular lesions and stated that the histologic picture of chancroid is characterized by the absence of specific changes. Greenblatt [5] considered the histologic changes as suggestive of chancroid, but not sufficiently distinct to permit the diagnosis on the basis of biopsy alone.

Our observations are based upon small specimens, most of which were taken for biopsy from fully developed or persistent lesions. No attempt will be made here to follow the sequences of formation in this lesion. However, the comparatively large number of specimens in this series, as well as 65 additional specimens of various genital lesions obtained from sources outside this study, made possible the development of certain general concepts. Our conclusions were further supported by independent bacteriologic studies of the same lesions.

The following description represents a composite picture as reconstructed from the study of numerous specimens, and refers to an active lesion of about 2 to 3 weeks' duration.

The lesion rarely extends more than 2 or 3 mm. in depth and under low magnification shows two or three zones which blend into each other (Fig. 1). There is generally a rather shallow surface zone of necrotic tissue representing the base of the ulcer. Below this is a fairly wide layer of edematous tissue with numerous blood vessels which are prominent and dilated. Their walls are thin and it is obvious that they are newly formed. In properly oriented sections the blood vessels display a vertical or palisade-like arrangement in their relation to the ulcerated surface. This zone leads into the deep layer in which there is a marked and diffuse cellular infiltration. Here the blood vessels, although still numerous, are less conspicuous and without any particular arrangement. The cellular infiltration fades into the adjacent edematous tissue where it persists around the dilated blood vessels.

On close observation the surface zone consists only of necrotic tissue, red blood cells, some fibrin and large numbers of neutrophilic polymorphonuclear leukocytes. Microorganisms are common and persistent search may reveal gram-negative coccobacilli between the cells of the surface zone, which are morphologically consistent with the Ducrey bacillus.

The mid-zone of the lesion is cellular. It is evident that the majority of cells in this zone are endothelial cells in varying stages of proliferation (Fig. 2). These outnumber all other cells. They are seen as apparently single elements or in small groups, as solid cords or buds originating from a capillary, and as already partially patent channels. Where the mid-zone joins the surface layer, the endothelial cells are

undergoing necrosis (Fig. 3); elsewhere they develop into capillaries. No microorganisms can be seen within these cells.

Marked endothelial proliferation is also seen in both the pre-existing and newly formed vessels in which the endothelium tends to encroach upon the lumen. The process involves arterioles, veins, and capillaries (Fig. 4). Fibrinoid degeneration of the vessel wall, margination and infiltration by neutrophilic polymorphonuclear leukocytes, and sometimes thrombosis are seen near the surface layer (Figs. 3 and 5).

The interstitial connective tissue is edematous. The lack of significant proliferation of fibroblasts in the presence of the marked endothelial overgrowth is striking (Fig. 2). Toward the surface a moderate number of neutrophilic polymorphonuclear leukocytes are present, while plasma cells appear in the deeper portions.

The deep zone of the lesion displays a marked infiltration by plasma cells and less numerous lymphocytes. This infiltration is diffuse and not particularly perivascular. The numerous blood vessels show well formed and thick walls. Endothelial proliferation is less marked and is largely confined to the lumen of the vessels. No degeneration of vessel walls with leukocytic infiltration or thrombosis is encountered.

The tissue surrounding the lesion is edematous and shows some fibroblastic proliferation. The blood vessels are dilated and their endothelium is prominent. There is some perivascular cuffing with lymphocytes and plasma cells. The lymphatics are markedly distended with granular eosinophilic material, some fibrin, and varying numbers of white blood cells. Occasionally, fibrin thrombi are found in the lymphatics which then may show appreciable endothelial proliferation.

The epidermis at the edge of the ulcer is necrotic and more peripherally shows edema, acanthosis, and some polymorphonuclear leukocytic infiltration. There is no repair. The inflammatory cellular infiltration extends for some distance beneath the epidermis on the sides of the ulcer.

In lesions of about 1 week's duration the findings are similar. Here, however, the surface zone of necrosis and ulceration is wide as compared with the mid-zone or the deep layer. The mid-zone displays the same striking endothelial overgrowth without proliferation of fibroblasts which has already been described. Necrosis of the vessel walls with leukocytic infiltration and thrombosis is frequent. The deep zone is poorly developed and there is relatively slight infiltration by plasma cells and lymphocytes.

In lesions older than 3 weeks, or when there is no further evidence of spreading, lymphocytes increase in the deep zone and eventually

outnumber the plasma cells. Sometimes distinct lymphoid follicles are formed. Plasma cells and, later, lymphocytes extend upward into the mid-zone. Healing takes place by proliferation of fibroblasts extending from the deep zone between the numerous blood vessels of the mid-zone (Fig. 6). Collagen is deposited, and some of the blood vessels, pinched off by connective tissue, undergo atrophy. By now the vessels have acquired thick walls. An increased number of endothelial cells line the vessels, but endothelial proliferation has largely subsided (Fig. 7). The base of the ulcer is filled in this manner and the epithelium at the edge of the lesion regenerates.

The same picture is seen not only in the primary lesion, but also in tissue from an area of auto-inoculation or from the wall of a ruptured bubo.

When the specimen includes tissue from all parts of the lesion the recognition of chancroid is not difficult. In many instances, however, the specimen is taken from the base of the ulcer and may show only the surface and mid-zone, with little of the deep layer and nothing from the edge or the surrounding tissues included. Even then the findings are sufficient to permit the diagnosis.

The demonstration of gram-negative coccobacilli in the section is not considered diagnostic, since it is subject to the same uncertainties as the direct smear.

Results

The histologic diagnosis of chancroid was made in 45 of 59 specimens taken for biopsy. The diagnoses in the remaining 14 cases were: lymphogranuloma venereum (7), granuloma inguinale (2), syphilis (2), and nonspecific inflammatory process (3). Since this investigation was intended to study chancroid and lymphogranuloma venereum, the preponderance of chancroidal infections in our biopsy material was to be expected. The routine material obtained from sources other than this series showed the usual incidence of venereal diseases.

The diagnosis of chancroid was made by biopsy in 45 cases, 35 of which were confirmed by culture. Biopsy yielded the correct diagnosis in all but one of the cases proved by culture. In this instance the Ducrey bacillus was cultured from the lesion, but biopsy showed granuloma inguinale with Donovan bodies.

There were 10 cases in which the culture for the Ducrey bacillus was negative but biopsy revealed chancroid. In all of these 10 cases the clinical picture was consistent with the diagnosis of chancroid.

Three patients in the group of 59 appeared clinically to have chancroid, but the specimens for biopsy showed only a nonspecific picture and the cultures were negative.

Comment

The histologic diagnosis of chancroid is based upon a number of findings, which taken individually are not characteristic but when found together may permit the diagnosis. There is the general architecture of the lesion consisting of an ulcerated surface and one or two deeper layers. The blood vessels display one of the most striking features. There is marked endothelial proliferation which in its diverse aspects occurs predominantly in the mid-zone. Here the endothelium in the various stages of overgrowth outnumbers all other cells. In the same area the lack of appreciable fibroblastic proliferation constitutes another important finding. The palisading of blood vessels, with degeneration of their walls and occasionally thrombosis, is part of the peculiar vascular pattern. There is finally the dense infiltration of the deep zone by plasma cells and lymphocytes with a gradual transition into the surrounding tissues.

The histologic picture can be differentiated from that of other genital lesions. It can be distinguished with reasonable accuracy from nonspecific inflammatory processes by following the criteria which have been outlined. Syphilis must be excluded, but its histologic picture is sufficiently well known to separate it from chancroid. Little is known about the histologic picture of the primary lesion in lymphogranuloma venereum, but in our experience it does not seem to bear any resemblance to chancroid. Granuloma inguinale is easily ruled out, for the general pattern is different and that diagnosis can be established by demonstrating the Donovan bodies.

The Ducrey bacillus was not cultured in 10 of the 45 cases in which a diagnosis of chancroid was made by biopsy. Two of these 10 lesions were on the upper vaginal mucosa and cervix, while in another instance the material was from a ruptured bubo. Because of the location and character of these lesions, the cultures were heavily contaminated. It has been our experience that the presence of other bacteria interferes with the growth of the Ducrey bacillus. The clinical appearance of the remaining 7 cases was entirely consistent with chancroid.

In this study we have encountered but one instance of mixed venereal infection in the same lesion. In this case, as already mentioned, the Ducrey organism was cultured from the lesion but biopsy showed the histologic picture of granuloma inguinale with typical Donovan bodies and no evidence of chancroid. Concurrent lymphogranuloma venereum was noted in 2 cases. In 5 other cases syphilis developed while the patients were under observation.

The diagnosis of chancroid by biopsy appears to have certain advantages. In our experience, securing tissue for biopsy is a simple procedure and does not interfere with healing of the lesion. For a variety of reasons the diagnosis is made more often by histologic examination than by culture. Although one tissue specimen is usually sufficient, two or three attempts to culture the lesion were sometimes necessary before the Ducrey bacillus could be identified. In fact, in 2 cases the Ducrey organism was obtained only from auto-inoculation lesions and not from the primary. Examination by biopsy seems particularly useful for those lesions which, because of location or long duration, are heavily contaminated by other bacteria. Present cultural methods usually require more than 24 hours of incubation, while our histologic diagnoses are routinely returned within this period. Securing tissue for biopsy, however, is a painful procedure in extremely small or early lesions and in such cases it is not a practical procedure. Fortunately, diagnosis by culture and smear is usually possible under such circumstances because of fewer contaminating bacteria.

In the course of this study the clinical diagnosis was changed in a number of cases by biopsy. Histologic examination not only confirmed other laboratory procedures but was also valuable when other methods failed. Early carcinoma, granuloma inguinale, and primary syphilis have been diagnosed on histologic evidence alone.

Conclusions

The histologic character of chancroidal infection was studied in specimens taken for biopsy from 45 patients. They formed part of a series of 125 cases selected for a special study of chancroid and lymphogranuloma venereum. In 35 of these 45 cases, the histologic diagnosis of chancroidal infection was confirmed by culture of the Ducrey bacillus obtained from the same lesions. The organism was not cultured from the other 10 cases, in which, however, the clinical picture was consistent with the diagnosis of chancroid.

The histologic picture of chancroid is sufficiently distinct to permit diagnosis and to differentiate this condition from other genital lesions. Useful features are the zonal character of the inflammatory reaction, the marked endothelial proliferation in the mid-zone, the meager fibroblastic response at the same level, and the dense infiltration with plasma cells and lymphocytes in the deepest zone. The advantages of biopsy in the diagnosis of chancroidal infection are such that this procedure is suggested as a practical diagnostic tool.

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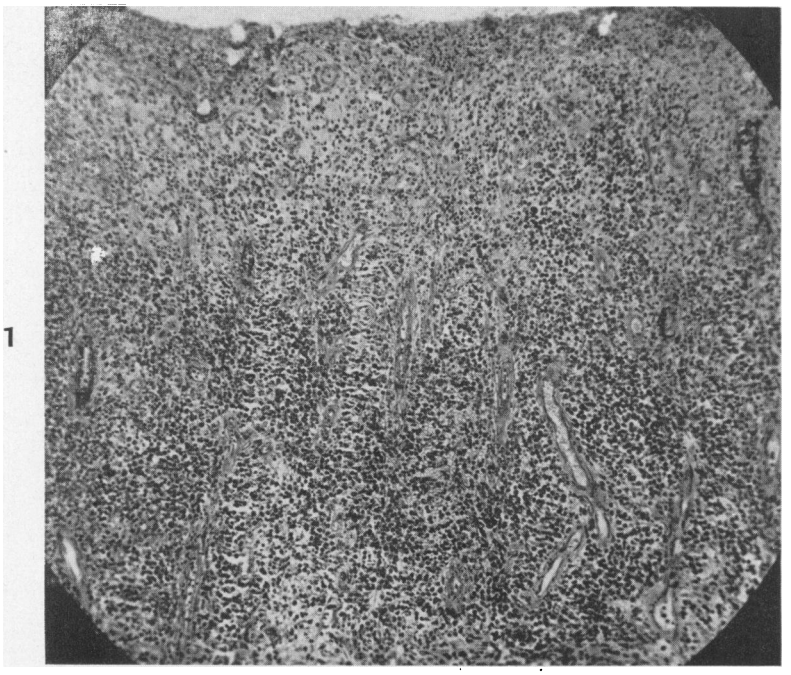
DESCRIPTION OF PLATES

PLATE 88

FIG. 1. The general pattern of the lesion. The shallow ulcerated surface zone is followed by the mid-zone and by the deep layer. In the mid-zone there are numerous blood vessels with some palisading. Some vessels show degeneration of their walls. Phloxine-methylene blue stain. × 80.

FIG. 2. Endothelial cells in varying stages of proliferation in the mid-zone. Endothelial cells outnumber all others, most of which are polymorphonuclear leukocytes. Phloxine-methylene blue stain. × 600.

FIG. 3. The transition of the mid-zone into the base of the ulcer where polymorphonuclear leukocytes predominate. The small blood vessel in the center shows degeneration of the wall and early thrombus formation. Below and to the right of the vessel is a small group of endothelial cells undergoing necrosis. Phloxine-methylene blue stain. × 550.



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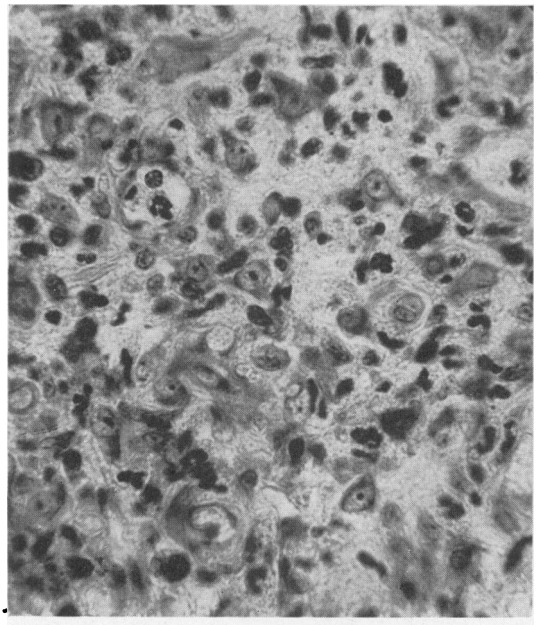

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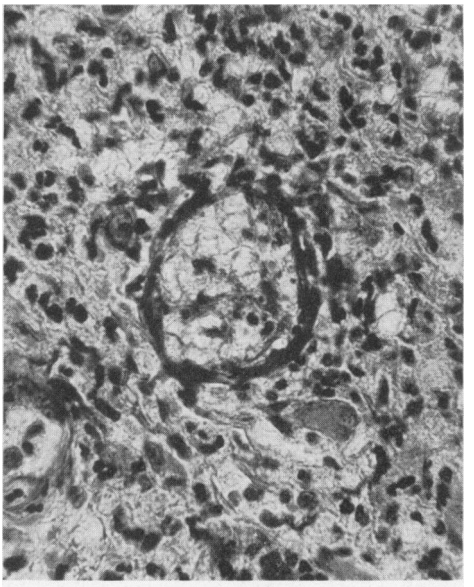

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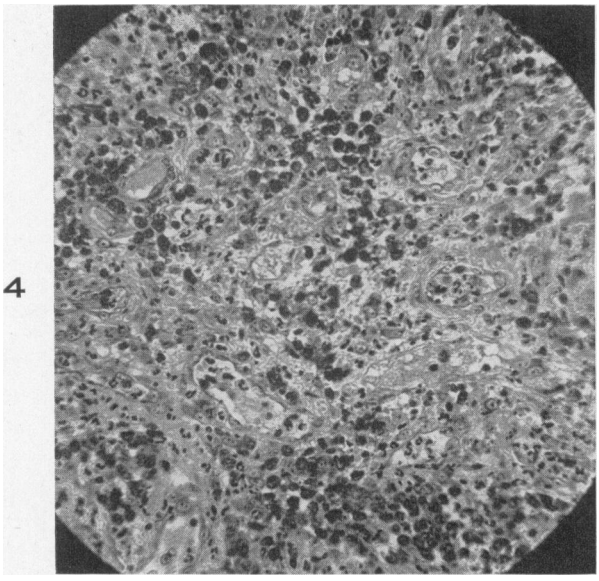
PLATE 89

FIG. 4. The numerous blood vessels in the mid-zone of the lesion showing proliferation of their endothelial lining. Phloxine-methylene blue stain. × 230.

FIG. 5. Another small artery of the mid-zone near the base of the ulcer with leukocytic infiltration of the wall and beginning fibrinoid degeneration. Phloxine-methylene blue stain. × 550.

FIG. 6. The mid-zone in a healing lesion of 28 days' duration. Of note are the thick walls of the blood vessels, the increase in interstitial connective tissue, and the lymphoid and plasma cell infiltration. Phloxine-methylene blue stain. × 200.

FIG. 7. Higher magnification of the lesion shown in Figure 6. Some vessels have become atrophic and consist only of small whorls of connective tissue. Phloxine-methylene blue stain. × 420.



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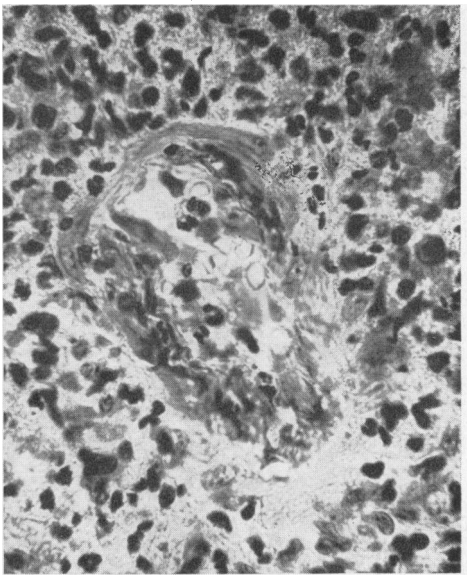

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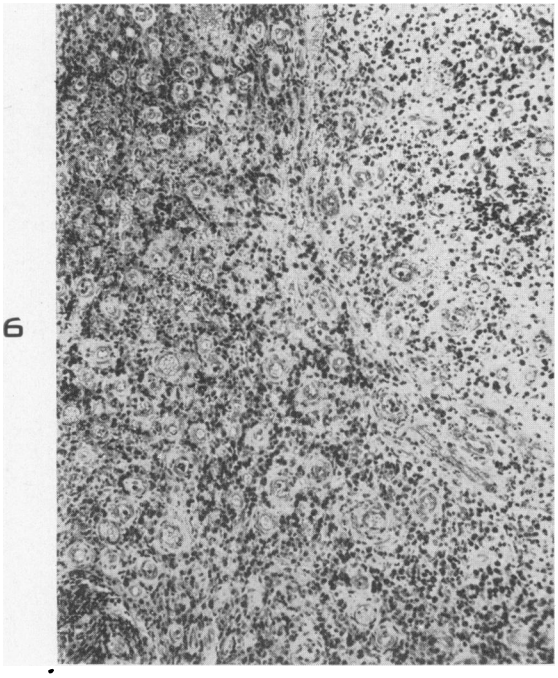

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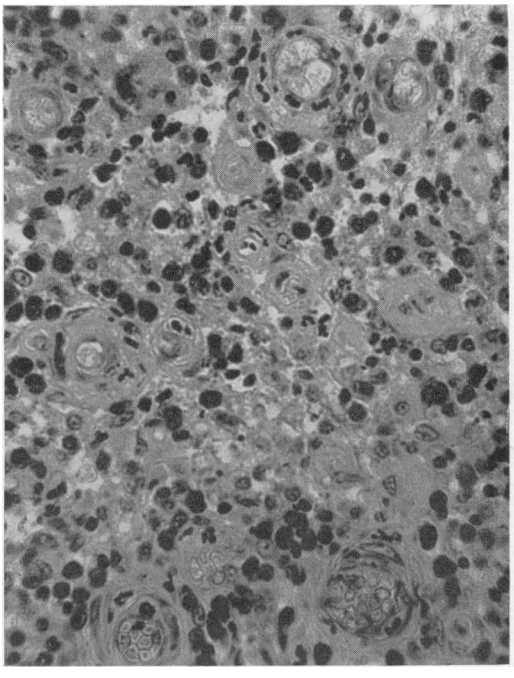

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