REPRINTED ARTICLES

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LEPRA BUBALORUM

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[Heretofore rat leprosy has been the only widely known disease of lower animals at all analogous to the human affection, and much attention has been paid it in connection with the study of leprosy despite decided differences in them. In 1934 there appeared a monograph under the foregoing title, describing a condition of the water-buffalo observed in the Netherlands East Indies which both in its gross manifestations and in the laboratory findings, bacteriological and histological, seems fully to justify the name given it. This monograph, containing 234 pages and 22 plates with 37 illustrations, was published both as a thesis for the degree of Doctor of Veterinary Science at the University of Utrecht, and as Veeartsenijkundige Mededeelingen No. 81 of the Department of Economic Affairs of the Netherlands India government at Batavia. Later a summary was published in the Nederländsche Indische Bladen voor Diergeneeskunde 46 (1934), No. 5. The matter seems of such interest in connection with the study of human leprosy as to make it desirable to reprint here a translation of this summary. Translation by Mrs. C. A. A. Mann, of Manila. —Editor.]

CONTENTS OF THE ORIGINAL PUBLICATION

INTRODUCTION

Under the title "Skin Tuberculosis (?) in Buffalos" Kok and Roesli, in 1926, called attention to a hitherto unknown disease in the water-buffalo which they had observed in three animals in Semarang. In this report they made the suggestion that it might be a kind of leprosy.

Between August, 1925, and November, 1932, with much difficulty eight new cases were traced. The study of these is described in this report. The disease was provisionally designated as "skin-nodule disease of buffalo." Between March, 1933, and April, 1934, judging from smear preparations that have been submitted, this diagnosis could be made in ten more animals.

OCCURRENCE

The total of 21 cases that have been found in Netherlands India have come from the districts of Semarang, Serang, Cheribon, Batavia and Menado. The disease therefore occurs in the Celebes as well as in Java, and it is to be expected that with increased familiarity and systematic search many more cases will be discovered.

CLINICAL OBSERVATIONS

Principal manifestations.—The most conspicuous phenomenon is the presence of skin nodules, measuring from about 5 to 60 mm. in diameter, which are sometimes so close together that they form large conglomerates. These nodules are hard in consistence, though the largest of them sometimes are more or less obviously fluctuating. There also occur small infiltrations of the skin, not raised or only

very slightly. The nodules may ulcerate, though this process takes place very slowly; each time there is a loss of a superficial layer with the result that after a few years large nodules are reduced to the original skin-level. In the progress of the disease there may occur depigmentation and diminution of hair. The extension of the skin processes varies very much; as a rule it is limited to the occurrence of nodules of different sizes on certain parts of the body, without infiltrations, but the whole body may be covered with nodules and infiltrations. Two buffalos had nose affections as well as skin lesions; one of them had ulcers and granulations on the nasal mucous membrane.

The general health as a rule is good; the animals can be worked and their former owners did not consider that their value was affected by the disease.

In only two animals were acute skin phenomena observed in the course of an observation of almost three years; these buffalos had extensive skin lesions. There was a slight rise of temperature, which in the beginning was accompanied by a general erythema of the skin and general hyperesthesia, and after some days there remained many small, raised, hyperemic spots which disappeared entirely after 10 to 24 days. Since no new nodules or infiltrations arose from these eruptions, the relation between the nodule disease and these acute phenomena could not be proved, but it is very likely that they were related.

Course of the disease.—Apart from the acute condition mentioned, the course of the disease is essentially chronic. Only after years of observation can changes of the process be seen. The principal changes are the ulcerations and the very slow resorption of the lesions described, which is attended by changes of pigmentation and of hair growth. The onset of the disease has not been observed.

Tuberculin test.—This test, made by means of the ophthalmoreaction, was positive in three of the eight cases when bovine tuberculin was used.[1] One of these three cases was doubtful with the subcutaneous test and the others were negative. With avian tuberculin the reactions were all negative. In a few instances intradermal tuberculin tests were made on some of the small test animals that had been inoculated with material from buffalos, but these all yielded negative results.

[1] In one of these cases the positive reaction was untrustworthy on account of the chronic hyperemia of the conjunctivae.

BACTERIOLOGY

Micro-organisms in the lesions.—In the skin nodules there are always found acid-fast micro-organisms of similar type which show a peculiar grouping. They occur usually as solid rods, but one also finds dark red, round granules and fragments of bacilli that are not quite round but angular, the coccothrix form. The rods and granular degeneration products are grouped in typical bundles, or globi, and these may assume large dimensions and be visible with low microscopic enlargement. This appearance is so characteristic that the diagnosis can be based on these findings alone.

In certain cases counts and measurements were made in order to get an idea of the number, size and composition of the globi which occur in the smear preparations. This was done with the aid of an ocular micrometer, the large square of which had been divided into 100 small squares. The side of each of these squares corresponded to 8 microns in the preparation, and consequently the whole area of each with 64 square microns. By counting the number of squares occupied by a globus the surface could be calculated approximately; parts of squares were estimated. The results of two countings on specimens from nodules of Cases 5 and 6 (Buffalos No. 235 and 236) are given in Table 1. In this table the globi have been grouped according to the number of squares of 8 × 8 microns that they covered, and have been designated according to the appearance of the bacilli in them, as follows: A, solid rods; B, coccothrix forms; C, dark granules and acid-fast grains. Combinations of these are also indicated. The smear preparations had been made by spreading two scrapings from nodules (the sizes of which were from 20 to 25 mm.) over a round spot in the center of the slide. Staining was by the Ziehl-Neelsen method. The number of globi to be counted and measured was so great that it was impossible to examine the entire preparation, but with the aid of the ocular micrometer it was approximately calculated which part of the entire preparation had been examined. About one-ninth of the smear from Case 5 was examined, and one-third of that from Case 6. The results give an idea of the particularly large numbers of bacilli which occur in these nodules, since in most globi they are innumerable.

These acid-fast micro-organisms also occurred in the lesions of the nasal mucous membrane, and in one of the five animals that came to autopsy they were also found in the lymph nodes of the

regions affected by the skin processes. They were never found in either the blood or the internal organs.

Attempts at cultivation.—Extensive series of cultures were made in an attempt to cultivate the organism. The tubes were observed for as long as two years, but the results were negative.

The inoculation of the media was done directly, as well as after preliminary treatment of the material with 5 or 10 per cent sulphuric acid. For the inoculation of liquid media, parts of nodules were cut into pieces and planted. Many attempts were also made to cultivate the organism under anaerobic conditions, in tubes filled with illuminating gas.

The culture media selected in the first place were those suitable for the organisms of tuberculosis and paratuberculosis. Others were used which, according to certain authors, would have yielded positive results in leprosy cases. Special media were also prepared with raw egg yolk, and with raw skimmed milk which had been filtered free of germs; sterilization by heating was avoided, so that these foodstuffs were kept in their original natural state.

TABLE 1.—*Globi in smear preparations from skin nodules, grouped according to the appearance of their contents.*

Contents of the globi	Bacilli lying isolated[a]	Number of globi, grouped according to number of areas 8 × 8 microns								
		-1 area	1-2 areas	2-4 areas	4-8 areas	8-16 areas	16-32 areas	32-64 areas	+64 areas	TOTAL
CASE 5										
A	76	122	19	5	—	1	—	1	—	148
B	6	3	—	—	—	—	—	—	—	3
C	—	16	9	4	1	2	—	—	—	32
A, B	—	18	5	2	—	—	—	—	—	25
A, C	—	35	11	7	6	2	2	2	1[b]	66
B, C	—	2	—	—	—	—	—	—	—	2
A, B, C	—	22	3	3	1	1	—	—	—	30
TOTAL -	82	218[c]	47	21	8	6	2	3	1	306
CASE 6										
A	33	62	7	4	3	—	—	—	—	76
B	6	2	—	—	—	—	—	—	—	2
C	—	61	17	10	2	3	1	1	—	95
A, B	—	4	3	1	—	1	—	—	—	9
A, C	—	30	6	4	2	—	—	—	—	42
B, C	—	—	—	—	—	—	—	—	—	—
A, B, C	—	—	—	—	—	—	—	—	—	—
TOTAL -	39	159[c]	33	19	7	4	1	1	—	224

[a] Bacilli lying in groups of 2 or 3 were also counted. Most of these were lying close to globi, from which they had probably been smeared away.

[b] The size of this globus was 150 squares (see text).

[c] The greater part of these globi were smaller than one-half square.

Inoculation experiments.—Attempts were made to infect buffalos, guinea pigs, rabbits, chickens, white rats, and white mice.

Four buffalos were allowed contact with four infected animals, and 3 were inoculated intra- and subcutaneously. Guinea pigs: 18 subcutaneously, 19 intramuscularly, and 13 intraperitoneally. Rabbits: 10 subcutaneously, 11 intramuscularly, 9 intraperitoneally, and 3 intravenously. Chickens: 7 subcutaneously, 9 intramuscularly, 8 intraperitoneally, and 4 intravenously. White rats: 19 subcutaneously and 20 intraperitoneally. White mice: 3 subcutaneously and 2 intraperitoneally.

These animals were observed for long periods—the guinea pigs and rabbits up to 24 months, the chickens up to 7 months and the rats up to 16 months. The results of this work were also negative.

Macroscopic appearances.—The shape of most nodules is round to oval, sharply demarked, firmly affixed in the surrounding cutaneous tissue. The smallest nodules are often more irregular in shape, and appear to be more infiltrative in the cutis. On cross section the nodules are yellow to brown, and they may be homogeneous or show fine or broader white strands of connective tissue, by which the tissue is divided into irregular islands. The consistency of the softer is somewhat firmer than that of a lymph-gland, while in others it is very hard. The cut surface is generally moist and glistening, like fat. Moreover, there often are points the size of a pin's head in which there is local necrosis and calcification. Larger centers of calcium occur, and as a rule have the aspect of a mortar-like substance. At the end of a four-year observation period there were found in one buffalo compact, amorphic calcified foci with a consistency like school chalk, but this was exceptional.

The surface may also show irregular, yellowish-white islands or streaks, owing to which the whole has a marbled appearance. As a rule this is the case only in larger nodules, and is caused by necrosis. The consistency is different from real caseation, being more compact and coherent. Breaking down of the tissue without ulceration occurs in some large nodules, in the form of a yellowish-white, watery fluid, with small tissue flakes (necrosis), with sandy-white pieces mixed in it (calcification).

The consistency of the granulations on the nasal mucous membrane is less firm than that of skin-nodules; the ulcers are covered with yellow pus and have distinct granulations at the sides.

Lymph glands have only exceptionally been enlarged without lesions being found in them.

PATHOLOGY

Microscopic technique.—Technical difficulties are met with in the histological examination. Buffalo skin with nodules is an ex-

traordinarily akward object to cut. However, the greatest difficulty is that, in making paraffin sections, the globi of bacilli as a rule almost completely disappear from the preparations; this also happens in a lesser degree in frozen sections. This difficulty is due to the fact that the globi are lying in round or oval spaces, embedded as it were in a fatty substance, and consequently they are easily lost in paraffin sections by treatment with fat solvents during embedding and staining. Under these circumstances dislocation of the globi in ordinary frozen sections is also easy. The search for a useful technique led to application of gelatine embedding, gelatine frozen sections and a suitable staining technique. These are fully described in the original publication.

Microscopic observations.—The skin affections seem usually to be located in the corium. In most cases a subepidermal zone seems to be left free; the width of this may vary greatly, from an extremely thin layer of collagenous tissue to a zone several millimeters wide. In case of ulceration the epidermis is affected secondarily; characteristic elements of the nodules do not occur in it. Subcutaneous lesions are rare. The skin infiltrations have the under surface of the epidermis for a base, leaving unaffected an extremely thin layer of tissue and radiating into the corium.

The nodules are granulomata, the general type of which can be characterized as a vascular tissue rich in cells, showing even with low microscopic enlargement many round to oval-shaped, vacuole-like spaces. The cellular tissue consists principally of large "granuloma cells" (see later) and fibroblasts; less numerous are lymphoid cells and still less numerous are polymophonuclear leucocytes. Acute inflammatory phenomena are not present. The border of the lesion is not sharp, and there is no marked reaction of the surrounding tissue. Collageneous fibers form a fine network in the granuloma, and connective tissue strands—either primarily present or caused by fibroblast proliferation—often give to it the form of irregular islands. At the sides of the lesion these islands occur in the form of small radiating lines which penetrate into the surrounding tissue without causing acute inflammation. Necrosis occurs in this general granuloma type of lesion in the form of irregularly scattered and irregularly shaped small areas which do not become prominent. As a rule these lesions, without demarkation zones, closely resemble living granular tissue.

The granuloma cells are macrophages which vary from the mononuclear form to multinucleate giant cells, among which are found those of the Langhans type. Morphologically all sorts of transition stages of these cell-varieties occur. In some of these cells the cytoplasm has a foamy structure, due to multiple vacuoles, but as a rule there is not more than one vacuole in a cell. Larger, vacuole-like spaces are no longer located intracellularly, but are surrounded by granular cells or other varieties. The numbers of vacuoles and vacuolar spaces differ very much in different cases. In case 7 (Buffalo No. 250) the number was very great; the number of small infiltrating cells was also greater than in the other cases.[2]

In gelatine frozen sections the foamy structure of the granular cells seems to be caused by fatty change of the cytoplasm. The vacuoles and larger spaces contain groups, bundles or globi of bacilli or granular products of their degeneration; moreover, they contain fat, and there are empty spaces infiltrated with gelatine. This fattening is a conspicuous phenomenon, as are also the bacillary globi which are so to speak embedded in the fat. The micro-organisms do not always fill the vacuoles completely, but are sometimes located eccentrically or arranged like a wreath.

Elastic fibers and small nerves in the granuloma become degenerated. Blood vessels and capillaries are lacking only in those parts where the granuloma cells lie closely pressed together.

Bacilli are as a rule located intracellularly in the granuloma cells. They do not occur in the lumen, endothelial lining, or wall of the vessels, or in the nerves. Whether they also occur in lymph spaces or the lymph vessels could not be determined from the morphological picture; that it is possible is evident from the fact that globi of acid-fast rods were found in the lymph-glands of one of the buffalos.

The relatively young portions of the granulomas arise perivascularly, the process proceeding along the loose connective tissue around the vessels, and also in the superficial layers of the corium around sebaceous glands, sweat glands and hair sheaths, which ultimately undergo atrophy and disappear. Neither granuloma cells

[2] In discussing size and numbers of the large vacuolar spaces the following have been used as standards: "small cavities," less than 25 microns; "large cavities," more than 75 microns. "Few" cavities, less than 20 per visual field at 54 × magnification (Zeiss ocular 2, objective AA); "many" cavities, more than 50 per visual field.

nor bacilli occur in the glands or the hair sheaths. In this case, though, it is true that globi were occasionally found in hair sheaths of a skin infiltration. If only a few granuloma cells are located at a distance from the granuloma these are, because of their fatty content, most easily recognized by fat staining. Connective tissue proliferation may in time, after some years, lead to fibrosis of the lesion.

Extensive necrosis may occur in the large nodules, but it may be absent. Collagenous fibers are long preserved in it, in the form of a coherent meshwork. Calcification always accompanies the necrosis. These changes may be attended by tissue softening, without the occurrence of purulent degeneration products. In the necrotic and calcified parts vacuolar spaces are still found. These are always filled with granular, degenerating micro-organisms, never with rods. Disturbances of circulation and nutrition in the compact tissue of the large nodules is probably the cause of the necrotic processes, and not a toxic action of the micro-organisms.

The epidermis shows principally atrophy and changes of pigmentation. In case of ulceration the epidermis degenerates, together with the uppermost layer of the granuloma tissue, and subsequently a new epithelial covering is formed upon the active tissue, which contains bacilli and granuloma cells. A considerable amount of the superficial portion of the lesion can thus successively become necrotic and be discharged.

A peculiarity is the occurrence of great globi in large cavities. The content never consists of solid rods alone; as a rule the larger the cavity the smaller the number of rods. The largest of these cavities, observed exceptionally, was 975 × 780 microns. These could be seen with the naked eye; they contained only granular, degenerated micro-organisms. A particularly vacuolar construction of the granuloma tissue, caused by very marked fattening of the granuloma cells, was observed in Case 4 (Buffalo No. 229).

The picture of the lesions of the mucous membrane corresponds in the main with that of the skin lesions. The *membrana propria* and the superficial gland layers are chiefly affected. Glands and their ducts are displaced by granuloma tissue. Neither granuloma cells nor bacilli are found in the lumena of gland ducts, tubules or acini. The process leads to both deep ulceration and progressive granulation formed by the granuloma tissue itself.

Nature of the disease.—Proof that the condition described is an infectious disease, caused by a pathogenic acid-fast bacillus, could

not be established on biological grounds. The attempts at cultivation as well as the inoculation tests gave negative results. Consequently, the viability as well as the pathogenicity of the micro-organism have not been demonstrated experimentally.

In spite of all this it may be taken for granted that this organism is the caustive agent of the disease. It was found in all cases in a total of 21 animals, including those examined by Kok and Roesli, and those with corresponding clinical phenomena from which smear preparations have been submitted for examination. The peculiar grouping and the conspicuous granular degeneration products are characteristic features by which the organisms can always be recognized. Moreover, as has appeared from the histological findings in eight cases examined, the organism causes a characteristic tissue reaction, a specific granuloma, the picture of which was similar in all cases except for some minor differences. From this we may conclude that this disease is an infectious one, caused by a pathogenic acid-fast bacillus. Any other possibility is inconceivable.

DIFFERENTIAL DIAGNOSIS

Only those diseases which are caused by acid-fast bacilli are considered here. Tuberculosis and paratuberculosis can be excluded. The cutaneous and subcutaneous nodules occurring in cattle in America are not comparable with the buffalo disease. Clinically worm-nodules may be mistaken for the nodules of the buffalo disease.

COMPARISON WITH HUMAN LEPROSY

In order to abbreviate the terminology, hereafter the nodule disease of buffalos and features belonging to it will be called "buffalo disease," "buffalo bacilli," "skin nodules," "granular cells," etc.

Nodular leprosy of man is considered for comparison. A short description of this disease is given in the original publication, as well as the histological features, and on the ground of published data and the histological examination of leprous material a comparison with the buffalo disease is made.

Lepra nervorum and the particular form of this called lepra maculo-anesthetica are left out of consideration. The examination of the buffalo disease has not indicated clearly that affections of neurogenic nature are present, with the exception of the loss of nerves in the granuloma. No maculo-anesthetic spots were seen,

though irregular depigmentation occurred, but this was never symmetrical and anesthesia could not be demonstrated. This, of course, would be difficult to determine in the buffalo. Loss of hair was also observed. These symptoms, which occur in human leprosy, are considered to be of neurotrophic nature. With brief mention of the occurrence of depigmentation and loss of hair as symptoms, the cause of these changes is left undecided.

Localization of the skin nodules of the buffalo disease corresponds with nodular leprosy. In general the lepromata seem to be flatter, and large lepromata seem to be of less frequent occurrence than large nodules. The diffuse thickenings (facies leonina) caused by extensive infiltrations are not found in the buffalo disease, so that the nodules and conglomerates stand out more clearly. Klingmuller (1930) in his manual gives a picture of nodular leprosy with large nodules which shows the same picture (page 285). It is true that in the buffalo disease small local infiltrations are met with, and all transitional stages from small (miliary) lesions to larger ones (papules) and big nodules.

Other points of correspondence are the firm consistency of the nodules and their latent, painless character, and also the ocurrence in some cases of lesions of the nasal mucous membrane. Lymph glands are as a rule not swollen in the buffalo disease, and the examination of the smear preparations in four cases was negative. However, the histological examination made in one instance (Case 7), demonstrated that the lymph glands, even when not swollen, may contain bacilli.

The course of the process in both diseases is extremely slow. In general the buffalo disease is less active and more benign than human leprosy; it corresponds to that form of leprosy in which the nodules may remain almost unchanged for long periods. This mild nature of the disease explains the usually negative result of the examination of the lymph-glands, and the fact that bacilli were never found in the internal organs. In leprosy the lymph glands seem always to be affected, and often also the internal organs and the nerves; pure skin leprosy is rare. In the buffalo disease, on the other hand, only the skin affection has as yet been found.

The deep ulcerations and the mutilations of leprosy are not found in the buffalo disease. In it the ulcerations have a benign course; as a rule they are limited to superficial loss of tissue, with speedy recovery. According to Unna this also occurs in leprosy. It

must also be recognized that in leprosy deep ulcerations and mutilations may arise from lepromata, but that the development of these is on a neurotrophic base; practically, they are not symptoms of the actual nodular leprosy but of neural or mixed leprosy.

The acute skin phenomena with a brief period of moderate fever and skin erythema observed in Cases 5 and 7 correspond to descriptions of acute, intermittent processes of leprosy in which, also, the development of lepromata from the small erythematous infiltrations does not always occur.

Softening of the nodules, without ulceration, occurs both in nodules and in lepromata. Necrosis, in the form of coagulation-necrosis, is a frequent phenomenon in the buffalo disease, generally in the form of very small foci but sometimes occupying the entire nodules. It is said that this form of necrosis is not found in nodular leprosy but is found in tuberculoid leprosy. It is therefore not so much a fundamental difference as one of gradation.

Calcification in the buffalo disease occurs regularly, as soon as necrosis takes place. In leprosy calcification is seldom heard of.[3] It may be that in buffalos the general conditions for the deposition of calcium in necrotic foci are more favorable than in man; in cattle, too, the inclination to calcification of necrotic tissue is generally great.

The positive results of the ophthalmo-tuberculin test in some buffalos is in agreement with the fact that many persons with leprosy react positively to tuberculin.

The negative results of the many attempts to cultivate the organism and to inoculate animals experimentally constitute an important indication of the close relationship between the buffalo-bacillus and that of leprosy.

In the microscopic appearances of the bacilli there is very close agreement. The most important features are their grouping into bundles and globi and their granular degeneration forms. The bundle-like arrangement of the buffalo-disease bacilli does not suggest "cigar-bundles," but this term, commonly used in leprosy, is descriptive, as can be seen from photomicrographs and microscopic preparations. In drawings of leprosy bacilli, too, their location generally agrees entirely with that of the buffalo bacilli. The mas-

[3] Aschoff says that calcification in lepromas sometimes produces laminated chalk-masses.

sing of the latter into globi is similar to that of leprosy bacilli, and they even exceed these in the degree to which this occurs. The granular degeneration of the bacilli is also more marked; the relation of the rods and granules may be entirely lost. These phenomena, so peculiar to the leprosy bacillus, are thus found to a greater degree in the buffalo disease.

The size of the buffalo bacillus corresponds to that of the leprosy bacillus, as well as the acid-fastness, and especially the staining by the methods of Baumgarten and Klingmuller. From the viewpoint of differential diagnosis these staining methods have only a limited value.

Histological points of agreement are the vascular character of the granuloma tissue, except in those places where the cells lie closely massed; the immunity of the epidermis, except in case of ulceration; the development of the granuloma around the vessels and along the glands, hair-sheaths, and hair muscles; the atrophy of these skin elements within the granuloma; the disappearance of elastic fibers the disappearance of the small nerves from the center of the granuloma; the chronic character of the lesion; the occurrence, in addition to the granuloma cells, of many fibroblasts and of a relatively small number of lymphoid cells (especially in the form of perivascular accumulations), and in still smaller degree of leucocytes.

With all this, however, the tendency for proliferation of connective tissue seems to be greater than in leprosy, in consequence of which the lesion may become separated into islands of granuloma tissue and finally the entire lesion may become fibrotic.

Most important are the conspicuous vacuolization of the granuloma through globus-formation, and the development of fatty material in both the bacillus-containing vacuoles and the granuloma cells. The globi, both the crown and the wreath-like forms, embedded in fatty masses, agree entirely with those of leprosy bacilli except for the fact that their dimensions are often greater and that the granular degeneration is more marked.

The fatty change in and around these globi is also comparable with that of leprosy, though it cannot be said whether the fatty material is the same in both cases. However, the fatty change in the granuloma cells is generally less marked than in the leprosy-cell; it is sometimes limited to an appearance of "pollination" of the cytoplasm with fine fatty particles, and only exceptionally (Case 7) have the cells of the granuloma tissue in paraffin sections shown

actual vacuolation that is comparable with that of the Virchow lepra cells. The foamy structure of the granuloma cells in paraffin sections is always much more fine-meshed than the vacuoles in the leprosy cell, and cannot be compared with it.

In the Virchow lepra cell there occur fat, bacilli (sometimes granular degeneration-products of them) and large numbers of vacuoles. The bacilli occur in it as small bundles or globi embedded in the fat, but they are also located outside and between the "true" vacuoles (Herxheimer) and in fat. The elements which in this case can be seen next to each other in a cell, in and round numerous small vacuoles, is in the buffalo disease concentrated in one large vacuole. The wreath-like formation, embedded in fat, can here be compared with the leprosy bacilli that are around and between the vacuoles, and the empty space in it to the "true" vacuole. This concentration in one large vacuole also occurs in leprosy, but less commonly. Jeanselme speaks of it as the "ultimate stage" of the lepra cell. In the buffalo disease it is the rule. On the other hand, the lepra cell with many small vacuoles is not produced in the buffalo disease; the fatty granuloma cells in the material from Case 7, mentioned above, did not contain bacilli and consequently were not real "lepra cells."

The usual intracellular location of leprosy bacilli is also characteristic of the buffalo bacilli. The vacuoles containing globi are formed intracellularly, and only later do they assume such proportions that the intracellular location becomes impossible, entirely in conformity with leprosy. The still greater spaces principally filled with granular bacilli are not recorded in leprosy.

The buffalo bacilli were observed in hair-sheaths, as well as in the granuloma cells and "cavities," a fact which also holds in leprosy. They were never found in other cells, except occasionally in leucocytes; they were not found in the endothelium of blood vessels, which is generally observed in leprosy. This may explain why the buffalo disease has such a latent character and why intermediate acute attacks do not occur or are rare.

In the buffalo disease the granuloma cells sometimes change into Langhans giant-cells, a phenomenon which may occur in leprosy but is certainly not the rule.

As a technical detail it must be noted that in paraffin sections of the human leprosy material studied for comparison there occurred

vacuole-like cavities, measuring as large as 44 microns in diameter, which were empty or contained only an irregular bluish mass; in gelatin frozen sections they contained characteristic globi, entirely in conformity with the buffalo disease. Many bacilli had also disappeared from other locations in the paraffin preparations, including the Virchow cells. These technical features agree with those observed in the buffalo disease.

SUMMARY

In summarizing, it can be said that the resemblance between the buffalo and leprosy bacilli is very close, both as regards their individual morphology and the important feature of grouping. The tissue reaction on the part of the host is in many respects the same. The important pathognomonic vacuolation and fat-production are outstanding phenomena. The multivacuolar form of the Virchow lepra cell is not formed, but the univacuolar form is produced. In the buffalo disease coagulation necrosis, calcification and formation of Langhans giant cells occurs frequently.

The clinical phenomena of the buffalo disease and nodular leprosy also show close agreement.

The negative results of the biological examinations constitute important evidence of the close relationship between the organisms of these two diseases.

On account of the close agreement between human nodular leprosy and the buffalo disease, the name "lepra bubalorum"—buffalo leprosy—is given to the latter. Or, if it is desired to give special emphasis to the particular nodular feature of this disease as it has been observed it might be called "lepra tuberosa bubalorum"—nodular buffalo leprosy.

CONCLUSION

Lepra bubalorum is a chronic infectious disease of the water buffalo caused by an acid-fast micro-organism. It shows very close resemblances to human nodular leprosy. The question whether its causative organism is entirely identical with that of human leprosy, and whether mutual infection is possible, cannot be answered.

DESCRIPTION OF PLATES *

Figures 1 to 11 and 32 are from photographs made at the Veterinary Institute at Buitenzorg; Nos. 12 to 31 and 33 to 37 at Pathological Institute of the Veterinary Faculty at Utrecht.

PLATE 8

FIG. 1. Case 1. Nodules in left cervical and neighboring regions.

PLATE 9

FIG. 2. Case 8. Right lateral view. (See also Fig. 3.)

PLATE 10

FIG. 3. Case 8. Detailed view of lesions.

PLATE 11

FIG. 4. Case 4. Nodules in the right ear.

PLATE 12

FIG. 5. Case 5. Nodules on the udder and legs.

PLATE 13

FIG. 6. Case 5. Left lateral view, September 9, 1930. (See also Fig. 7.)

PLATE 14

FIG. 7. Case 5. Same as Fig. 6, May 1, 1931.

PLATE 15

FIG. 8. Case 6. Foreleg and thigh, September 9, 1930. (See also Figs. 9 and 10.)

PLATE 16

FIG. 9. Case 6. Same as Fig. 8, May 4, 1931. (See also Fig. 10.)

PLATE 17

FIG. 10. Case 6. Same as Figs. 7 and 8, November 4, 1932.

PLATE 18

FIG. 11. Case 7. Right lower part of abdomen, showing ulcerated nodules with crusts.

PLATE 19

FIG. 12. Border of a skin infiltration, showing extension of the lesion. (Paraffin section, hemalum-eosin, 22×.)

FIG. 13. Perivascular extension of the granuloma tissue in the corium. Fat stain, the fat appearing dark. (Gelatin section, 65×.)

PLATE 20

FIG. 14. Granuloma tissue with many vacuoles and vacuole-like spaces. (Paraffin section, hemalum-eosin, 22×.)

FIG. 15. Vacuoles and vacuole-like spaces occupying a large part of the field. (Paraffin section, hemalum-eosin, 300×.)

* The expense of producing these plates has been met in part by the author. —EDITOR.

PLATE 21

FIG. 16. Foci of the granuloma tissue around blood-vessels, glands and hair sheaths, with giant cells. (Paraffin section, hemalum-eosin, 65×.)

FIG. 17. Giant cells with beginning vacuole-formation. (Paraffin section, hemalum-eosin, 300×.)

PLATE 22

FIG. 18. Granuloma with formation of connective tissue. The epidermis is intact, with a subepidermal zone free of the granuloma. (Paraffin section, hemalum-eosin, 22×.)

FIG. 19. Broad bands of connective tissue, with remnants of granuloma tissue. (Paraffin section, hemalum-eosin, 22×.)

PLATE 23

FIG. 20. Granuloma with an irregular necrotic area in which there is calcification. Accumulations of lymphoid cells in the living tissue nearby. (Paraffin section, hemalum-eosin, 22×.)

FIG. 21. Macrophages near a necrotic focus. (Paraffin section, hemalum-eosin.)

PLATE 24

FIG. 22. Ulceration of a granuloma, with epithelial proliferation. (Paraffin section, van Gieson, 65×.)

FIG. 23. Atrophy and stretching of the epidermis. Fibrosis with remnants of granuloma tissue. (Paraffin section, van Gieson, 65×.)

PLATE 25

FIG. 24. Large vacuolar spaces, some of which are confluent. Necrosis and calcification. At the top, center, an accumulation of lymphoid cells. (Paraffin section, hemalum-eosin, 22×.)

FIG. 25. Extremely large vacuolar spaces. A focus of calcification at the right, top, surrounded by living granuloma tissue. (Paraffin section, hemalum-eosin, 22×.)

PLATE 26

FIG. 26. Granuloma cells with foamy structure of the cytoplasm caused by fat production. (Paraffin section, hemalum-eosin, 300×.)

FIG. 27. Vacuolation of the cells, caused by marked fat production. (Paraffin section, hemolum-eosin, 300×.)

PLATE 27

FIG. 28. General view of bacillary globi in a skin infiltration. Dark masses that are not globi are seen in the papilli (erythrocytes in capillaries) and in the hair sheath in the left top corner. (Gelatin section, stained for bacilli, 22×.)

FIG. 29. Bacillary globi in a hair sheath. The dark masses in the epidermis in the right top corner are not globi. (Gelatin section, stained for bacilli, 65×.)

PLATE 28

FIG. 30. Bacillary globi completely filling vacuole-like spaces. From some spaces the contents have been partly or entirely lost. (Gelatin section, stained for bacilli, 65×.)

FIG. 31. Showing bacillary globi, with empty spaces, in large cavities. From the two confluent cavities at the left the contents have been lost. (Gelatin section, stained for bacilli, 65×.)

PLATE 29

FIG. 32. A bacillary globus containing an eccentric empty space. (Smear preparation, Ziehl-Neelsen, ±1400×.)

FIG. 33. Globi with rods and granules. (Gelatin section, stained for bacillary fat, 700×.)

PLATE 30

FIG. 34. Globus of solid rods embedded in fat; the staining is not done justice in the photograph. (Gelatin section, bacilli stained for fat, 700×.)

FIG. 35. Globi, bundles and dispersed bacilli. (Gelatin section, stained for bacilli, 700×.)

PLATE 31

FIG. 36. Wreath-shaped globus of degeneration products of bacilli, embedded in fat. (Gelatin section, stained for bacillary fat, 700×.)

FIG. 37. Border of an especially large cavity filled with bacillary degeneration products. (Gelatin section, stained for bacilli, 700×.)

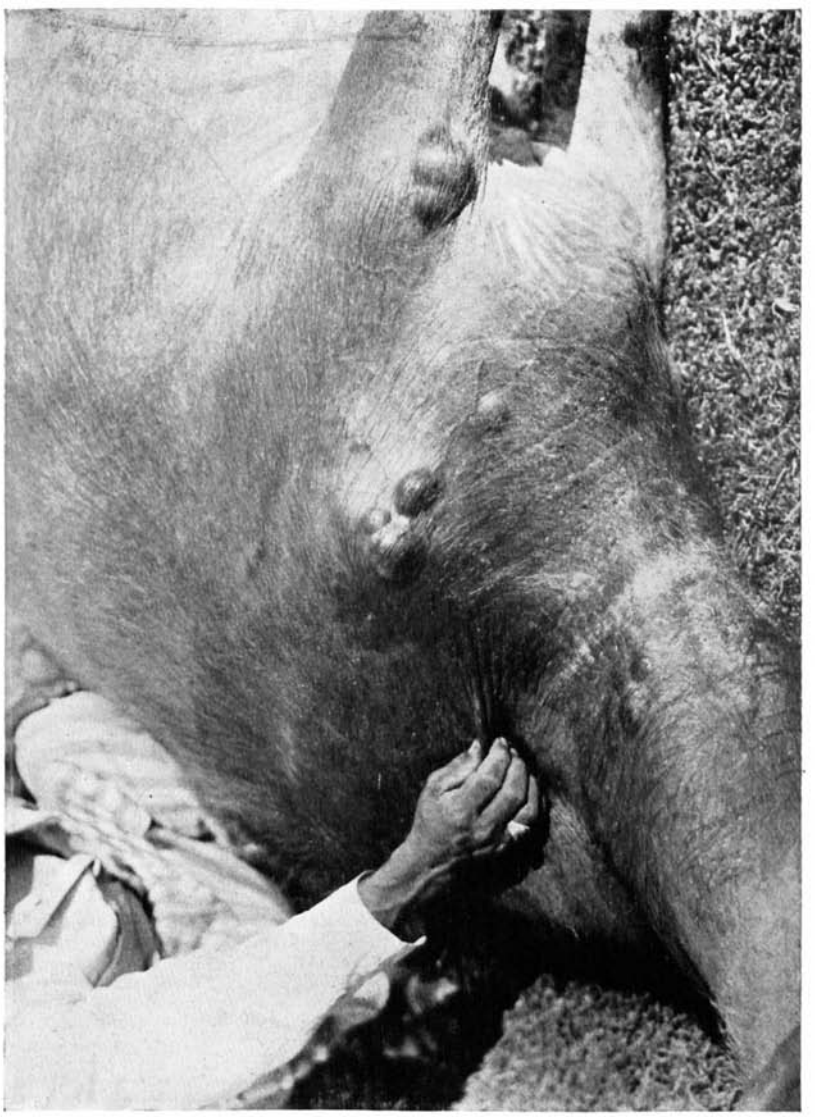

FIG. 1. Case 1. Nodules in the left cervical region.

PLATE 8

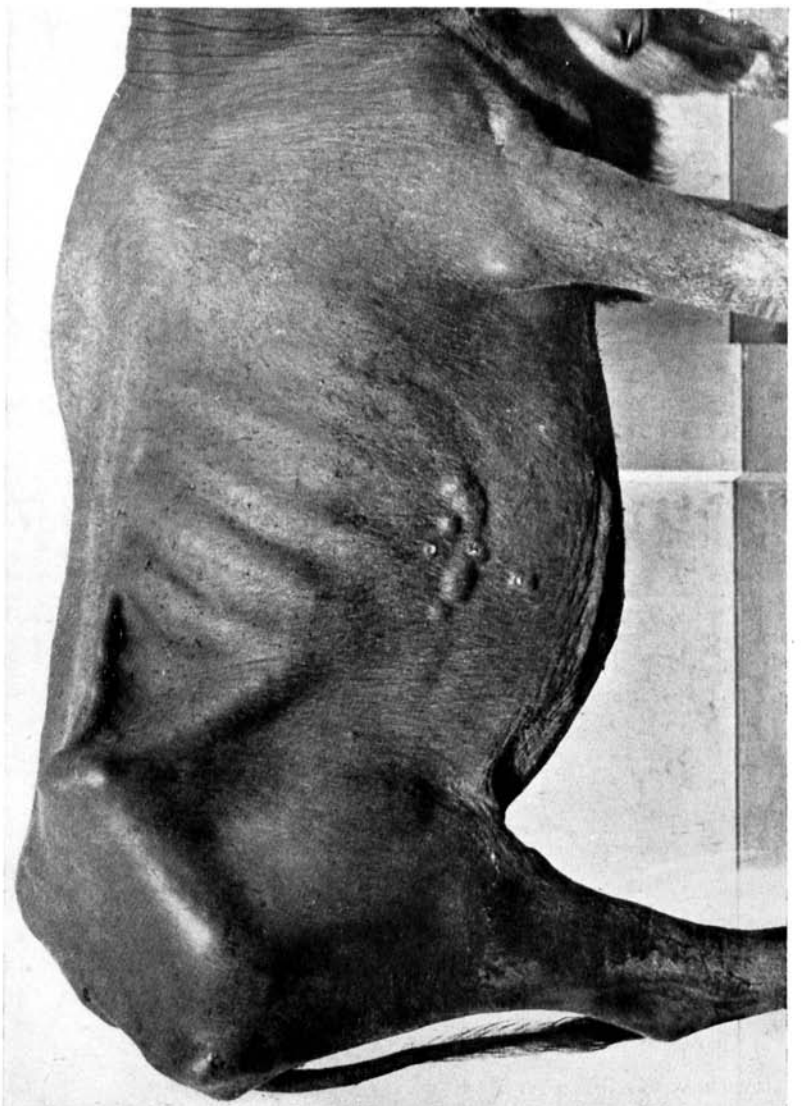

FIG. 2. Case 8. Right lateral view.

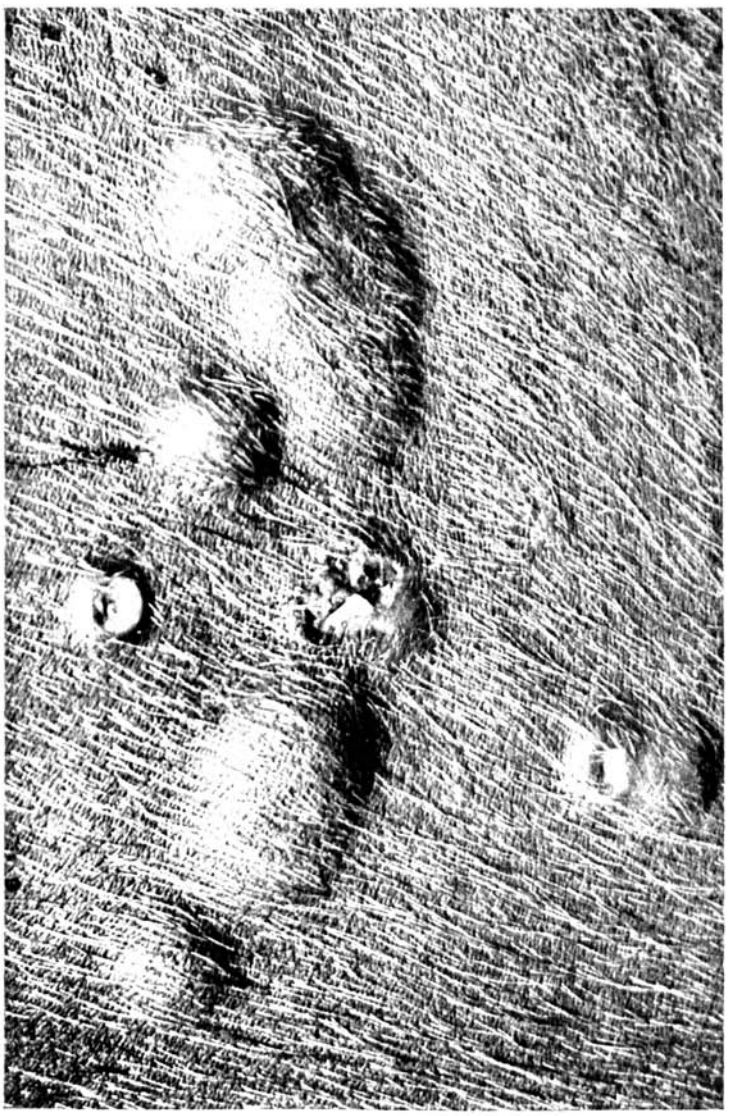

Fig. 2a. Case 2. Detailed view of Lesions.

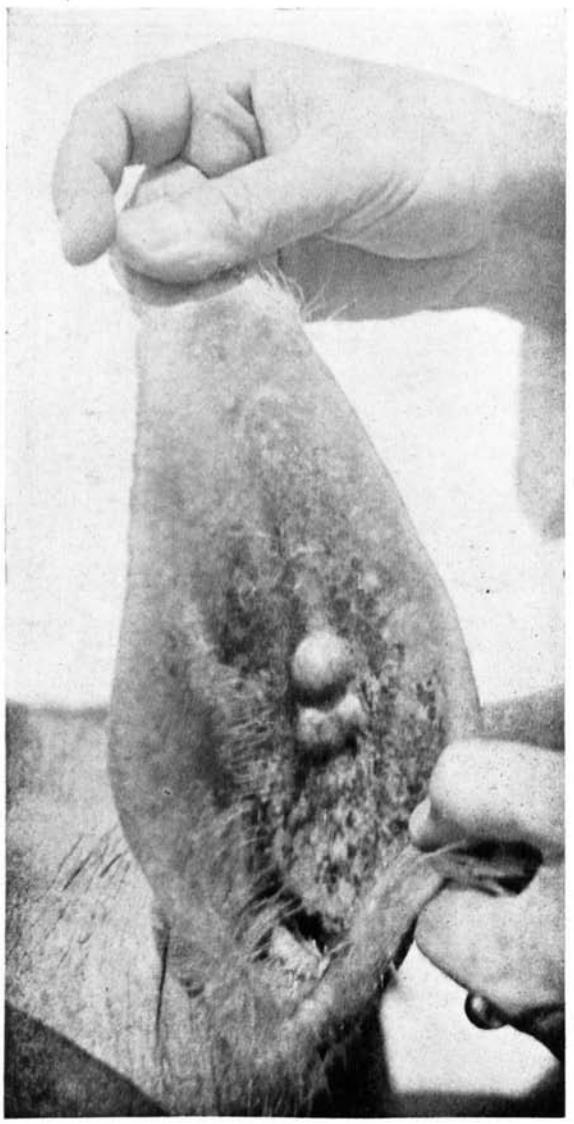

FIG. 4. Case 4. Nodules in the right ear.

PLATE 11

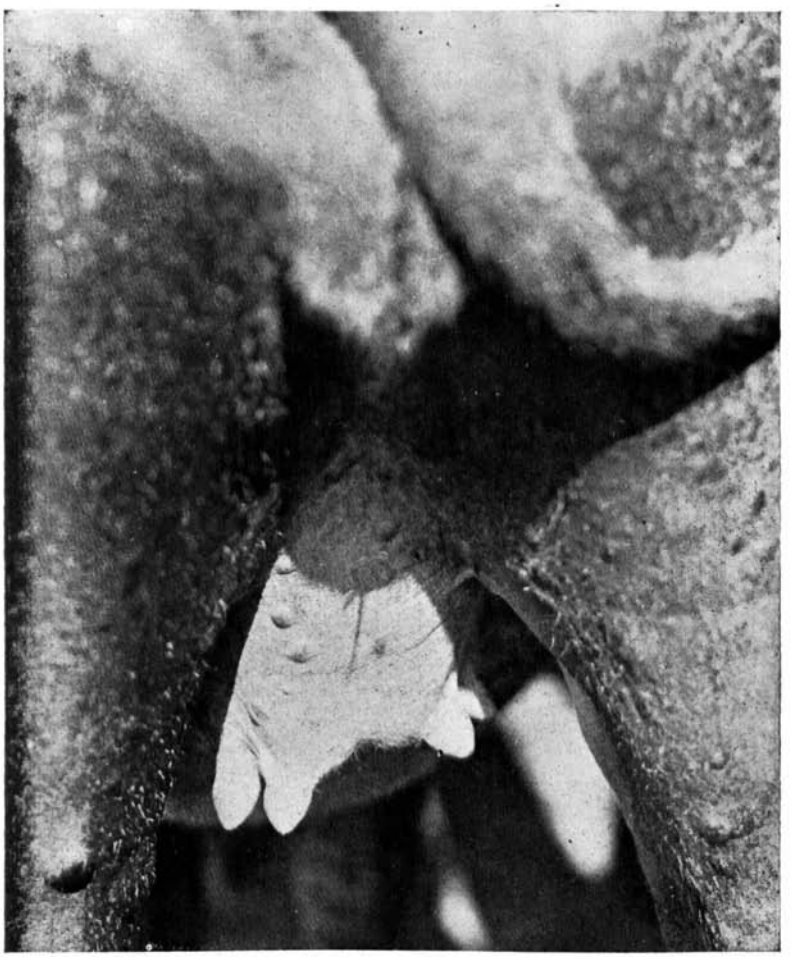

FIG. 5. Case 5. Nodules on the udder.

PLATE 12

Fig. 6. Case 5. Left side, September 9, 1950.

FIG. 7. Case 5. Same as Fig. 6, May 1, 1931.

PLATE 14

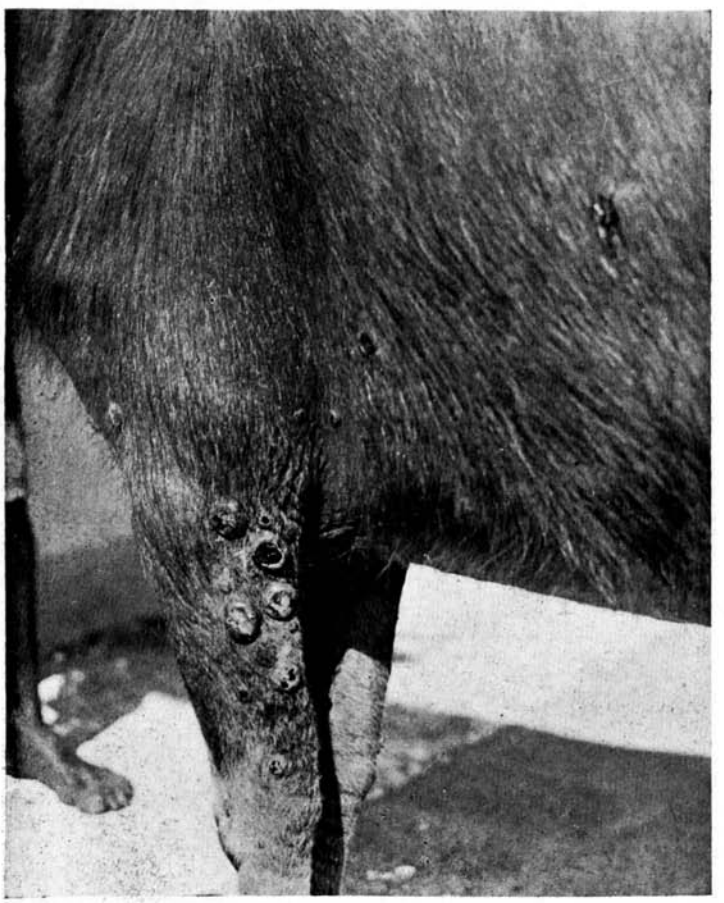

FIG. 8. Case 6. Foreleg and side, September 9, 1930.

PLATE 15

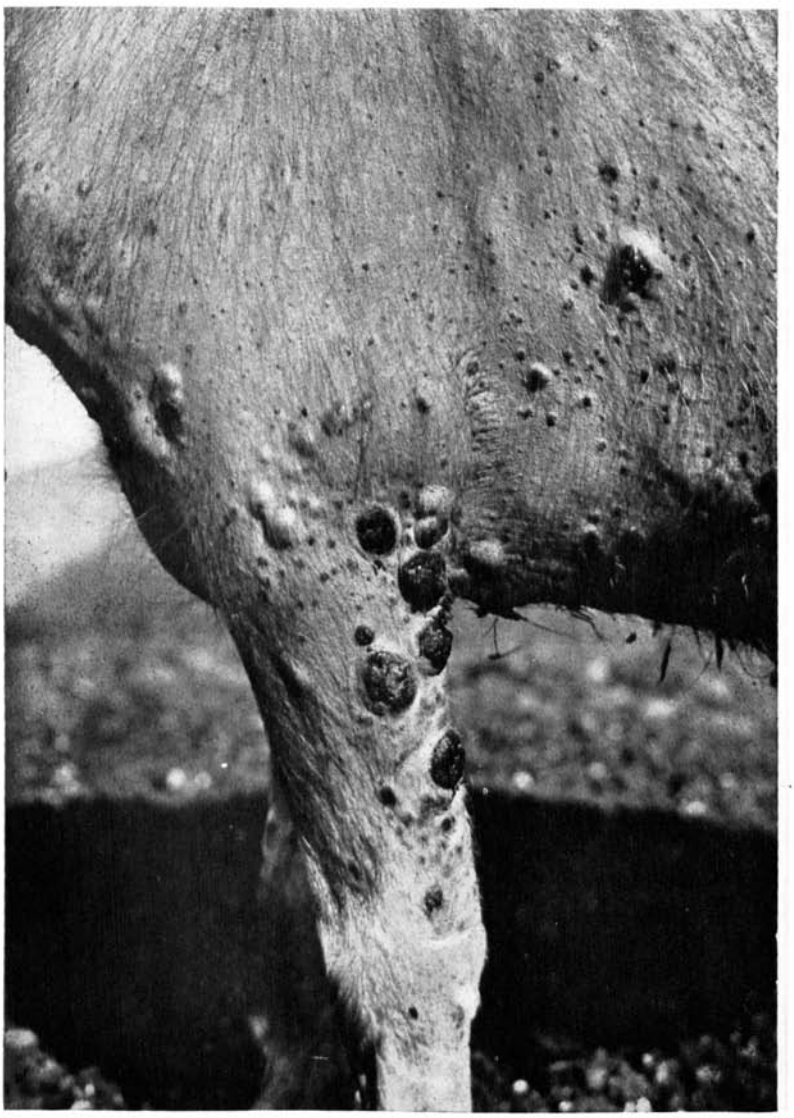

FIG. 9. Case 6. Same as Fig. 8, May 4, 1931.

PLATE 16

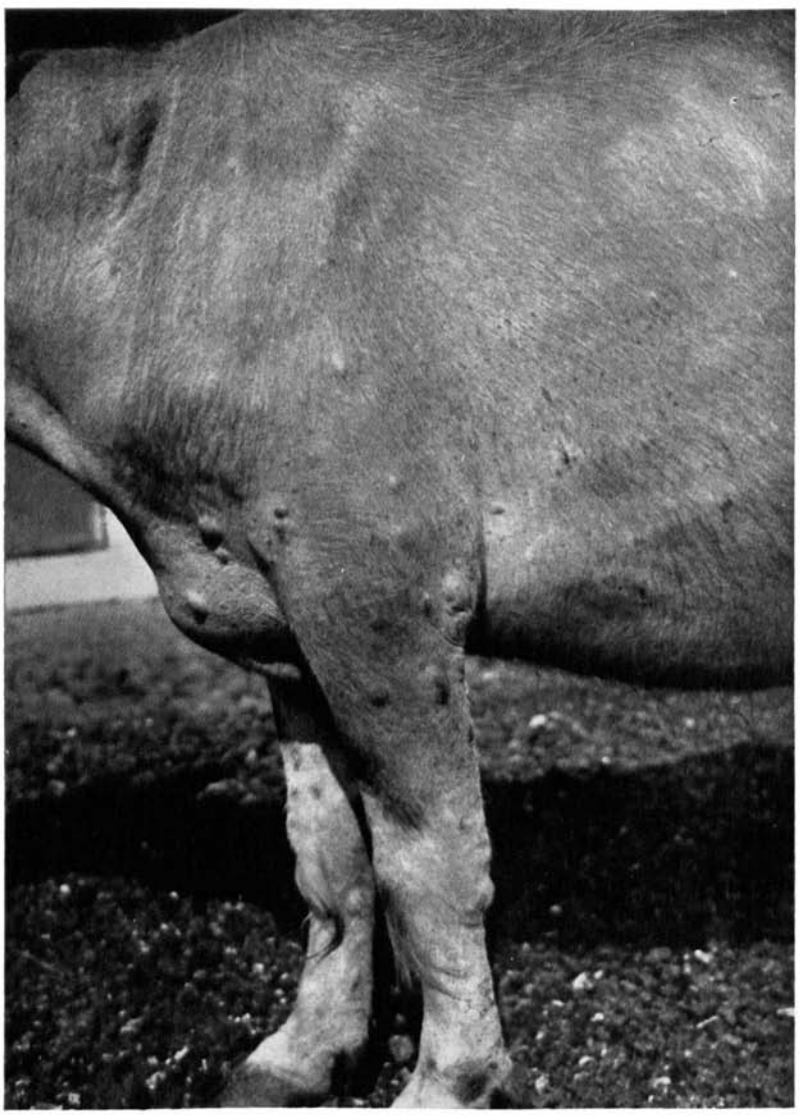

FIG. 10. Case 6. Same as Figs. 7 and 8, November 4, 1932.

PLATE 17

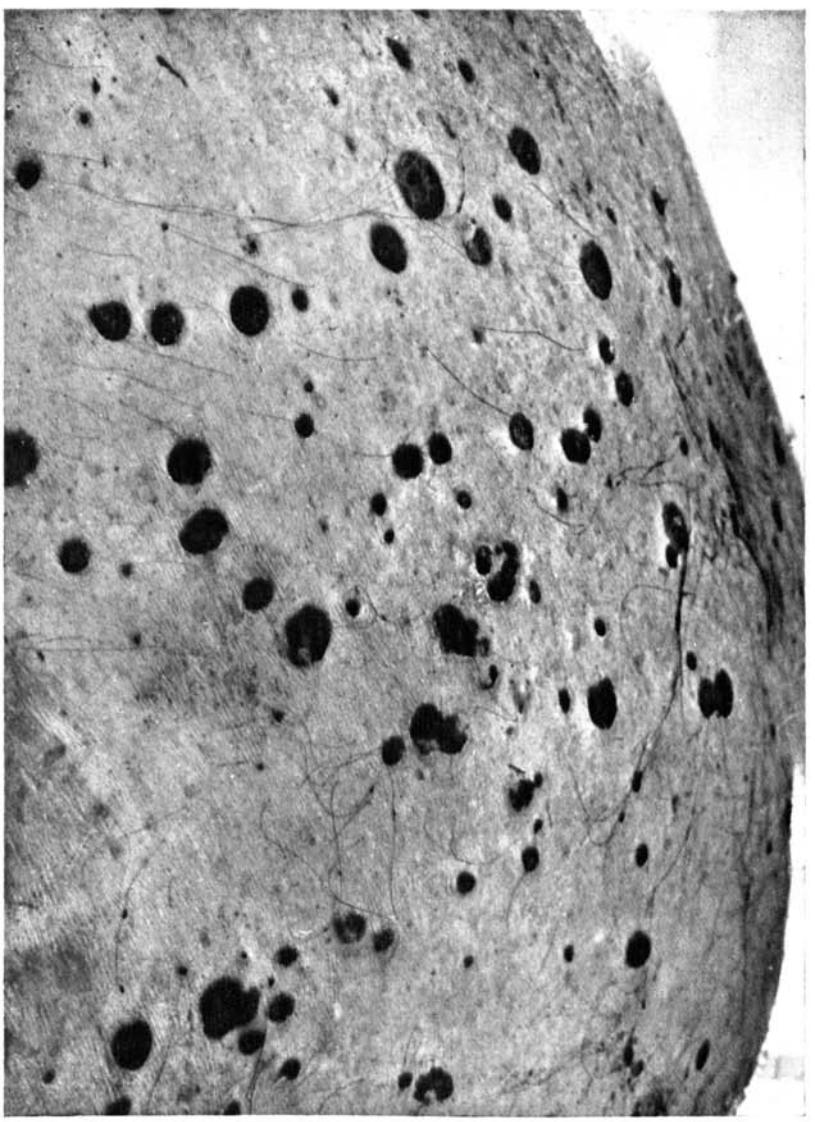

FIG. 11. Case 7. Right abdomen, ulcers with crusts.

PLATE 18

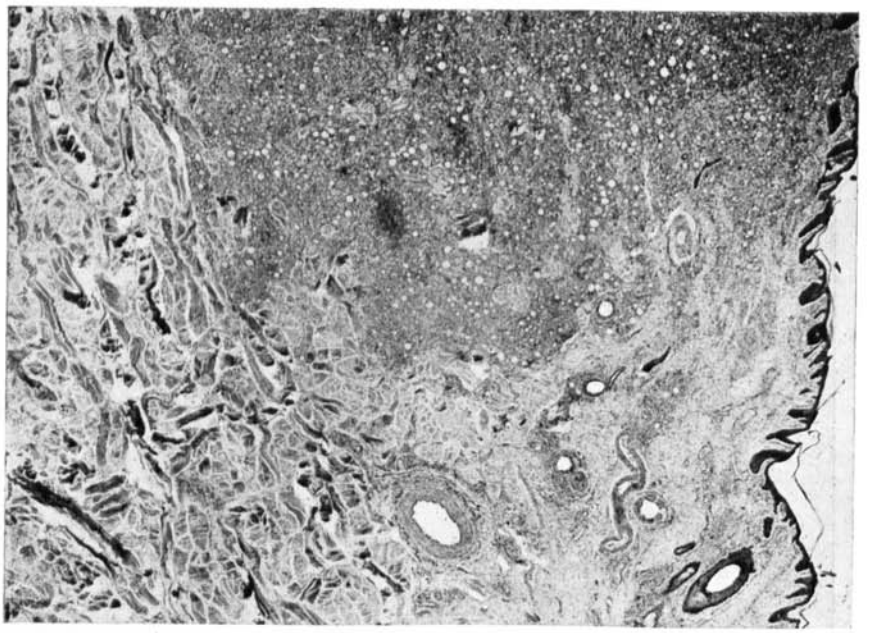

FIG. 12. Border of an infiltration, showing extension. 22×.

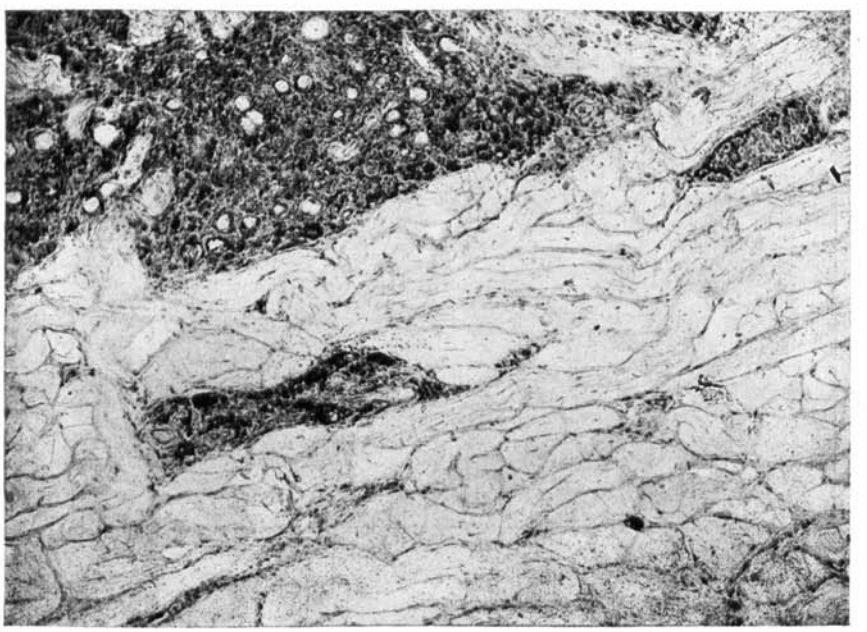

FIG. 13. Perivascular extension, fat stain. 65×.

PLATE 19

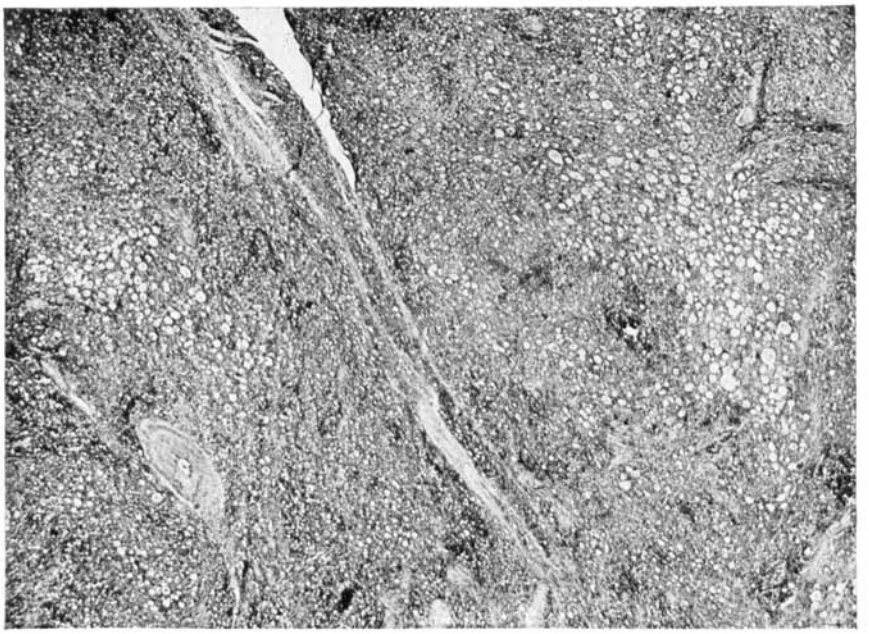

FIG. 14. Granuloma with many vacuoles. 22×.

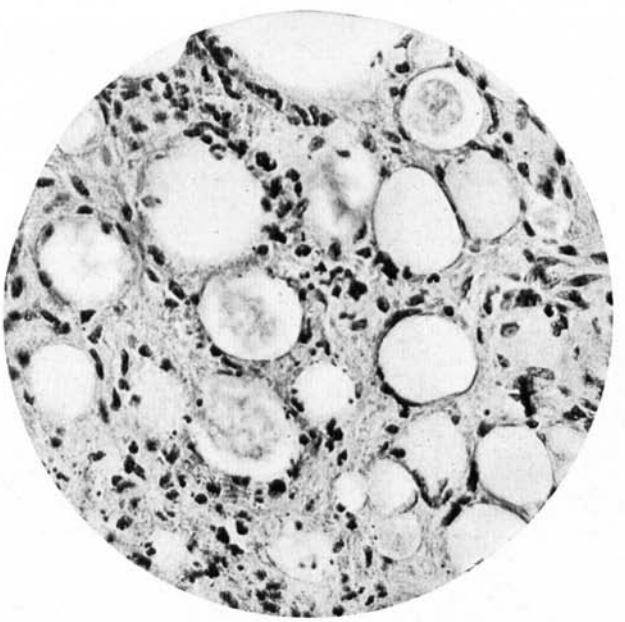

FIG. 15. Numerous, close-lying vacuolar spaces. 300×.

PLATE 20

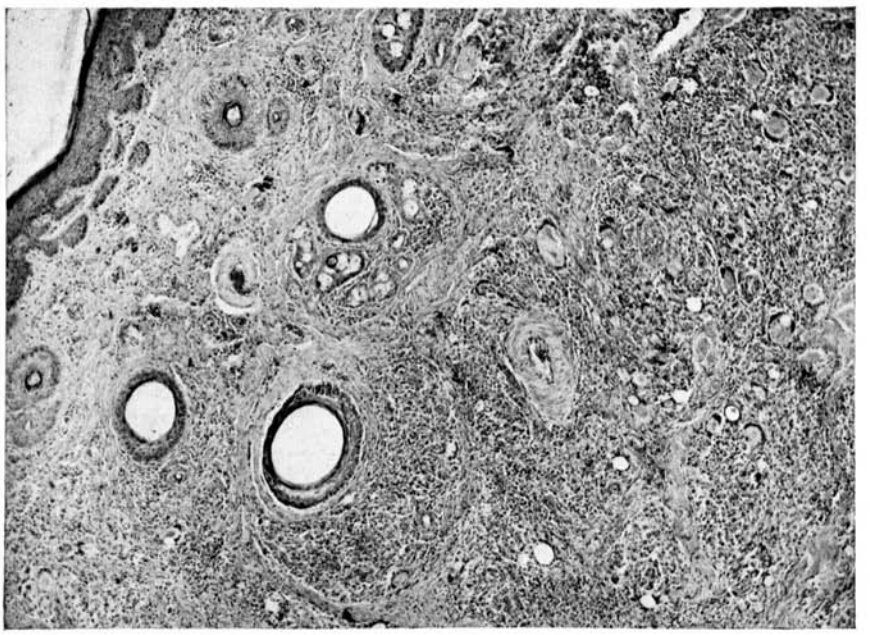

FIG. 16. Granuloma around vessels, glands and hairs. 65×.

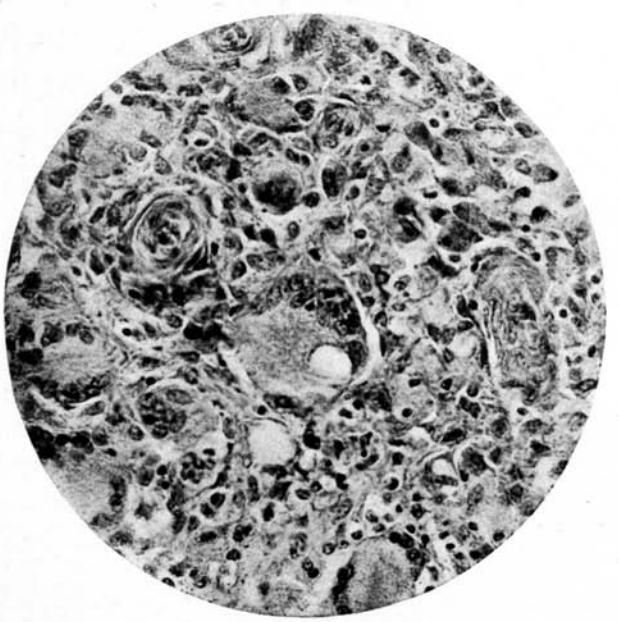

FIG. 17. Giant cells undergoing vacuolation. 300×.

PLATE 21

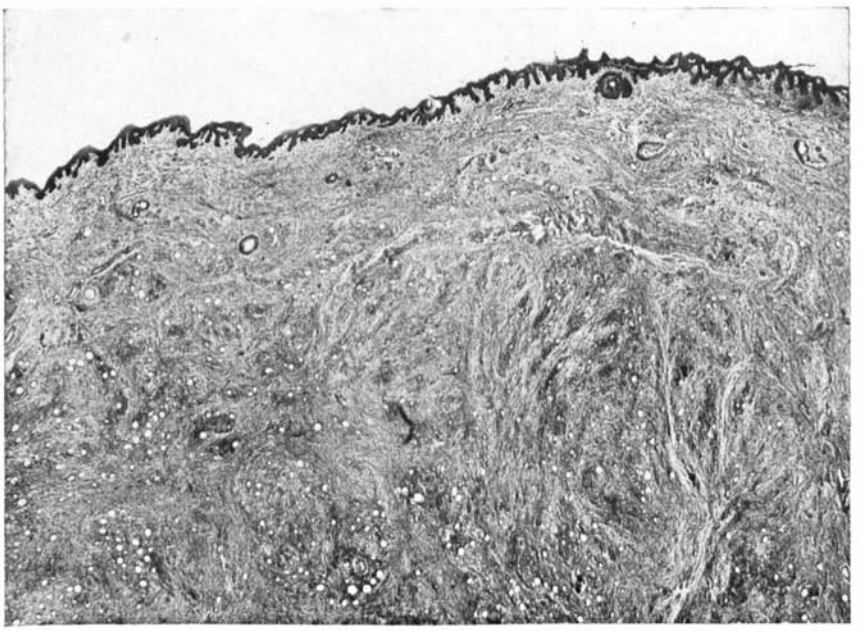

FIG 18. Granuloma undergoing fibrosis. 22×.

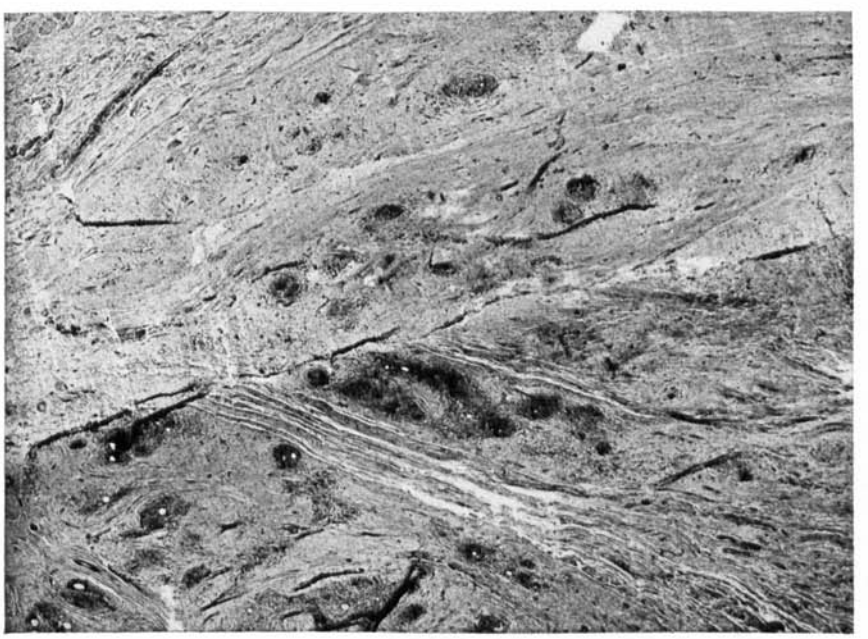

FIG. 19. Fibrotic lesion with remnants of granuloma. 22×.

PLATE 22

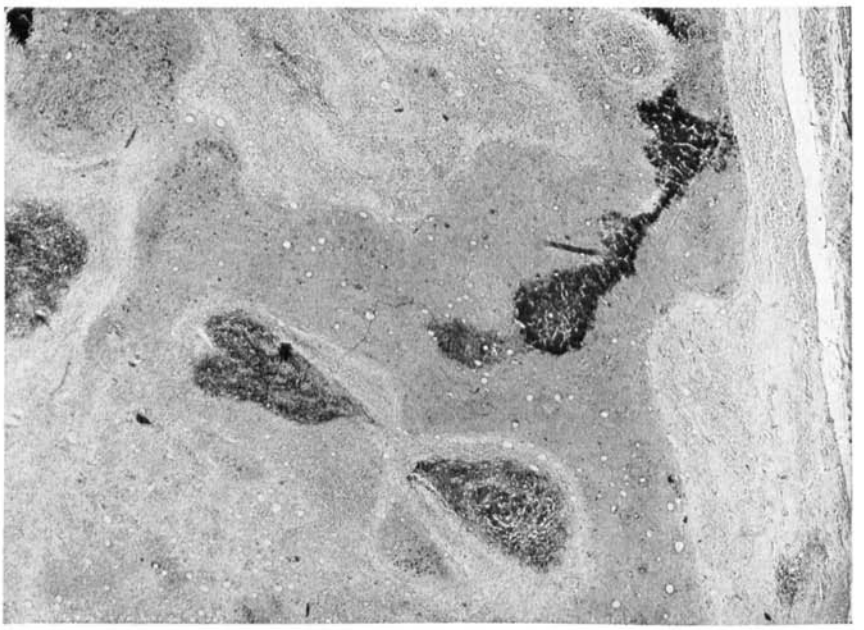

FIG. 20. Granuloma with necrosis and calcification. 22×.

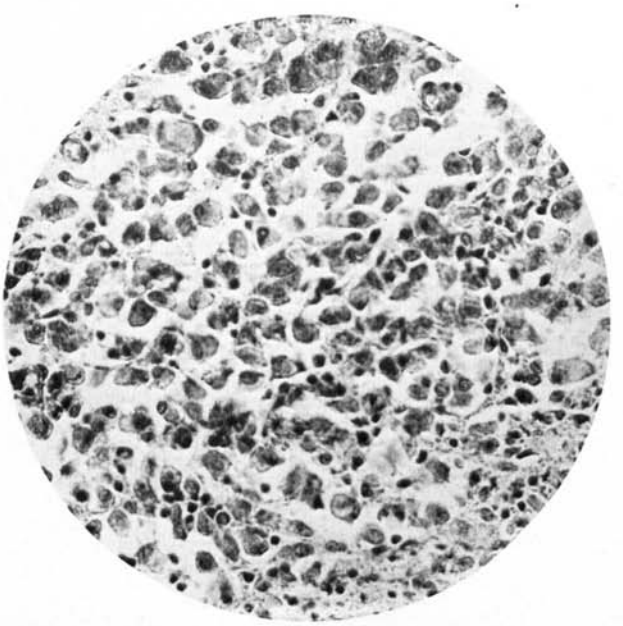

FIG. 21. Macrophages near a necrotic focus.

PLATE 23

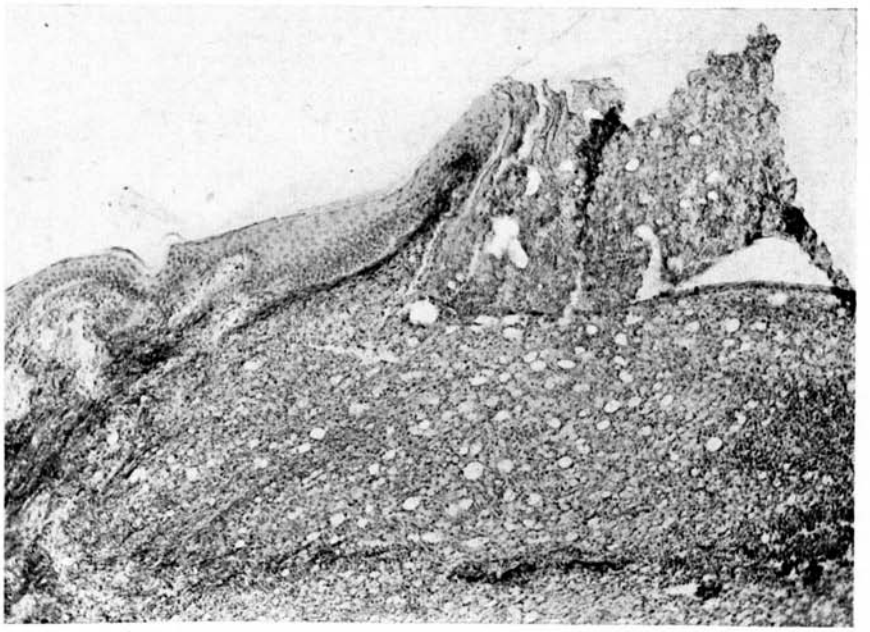

FIG. 22. Ulceration and epithelial proliferation. 65×.

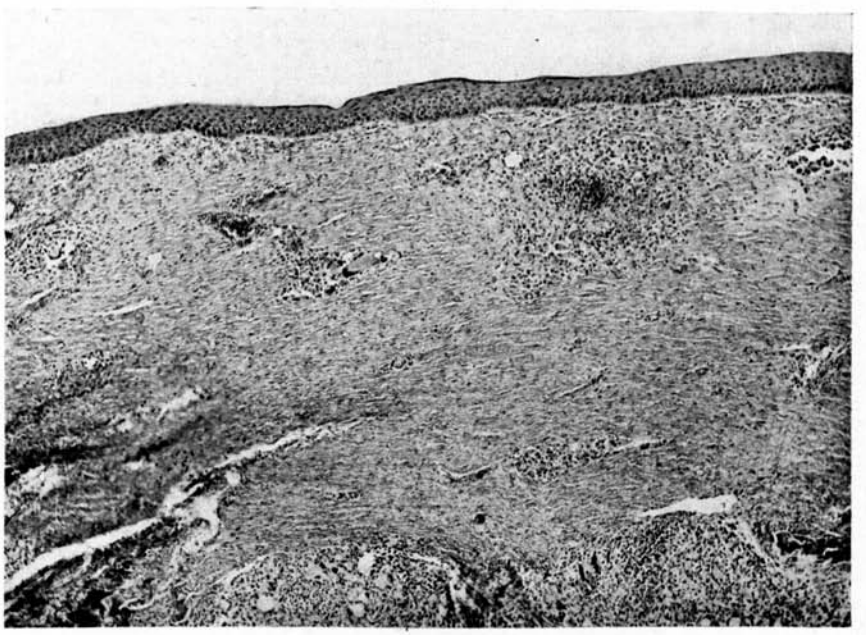

FIG. 23. Stretching of epithelium; fibrosis. 65×.

PLATE 24

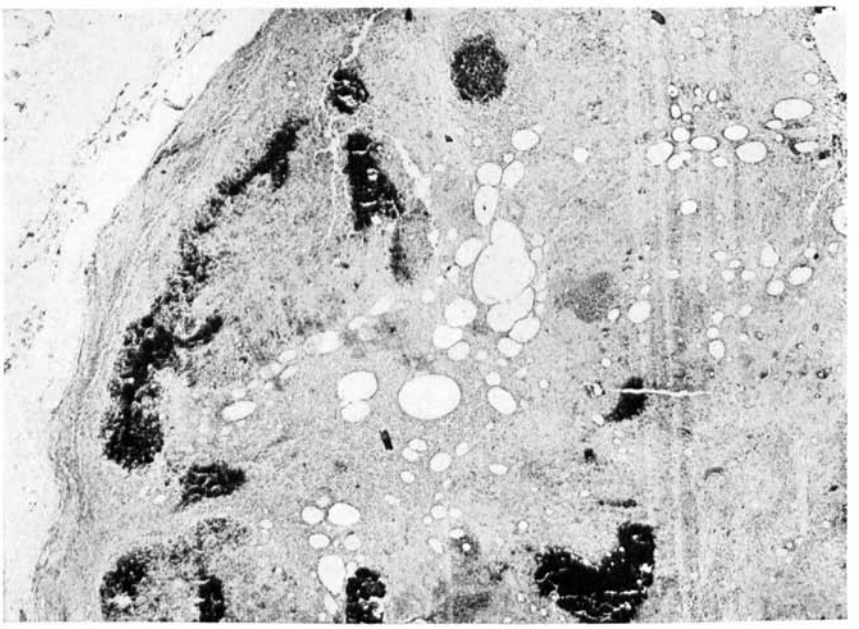

FIG. 24. Confluent spaces; necrosis and calcification. 22×.

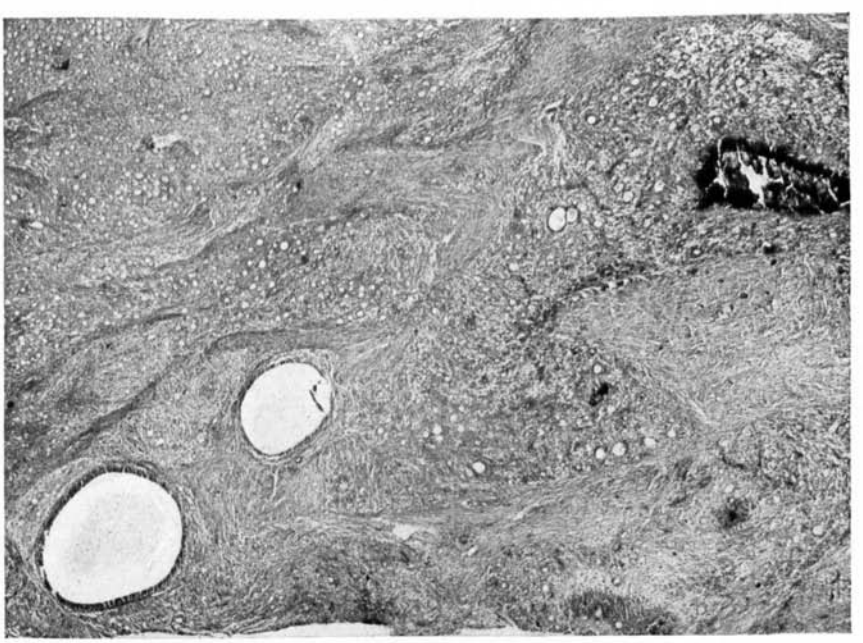

FIG 25. Extreme vacuolar spaces; area of calcification. 22×.

PLATE 25

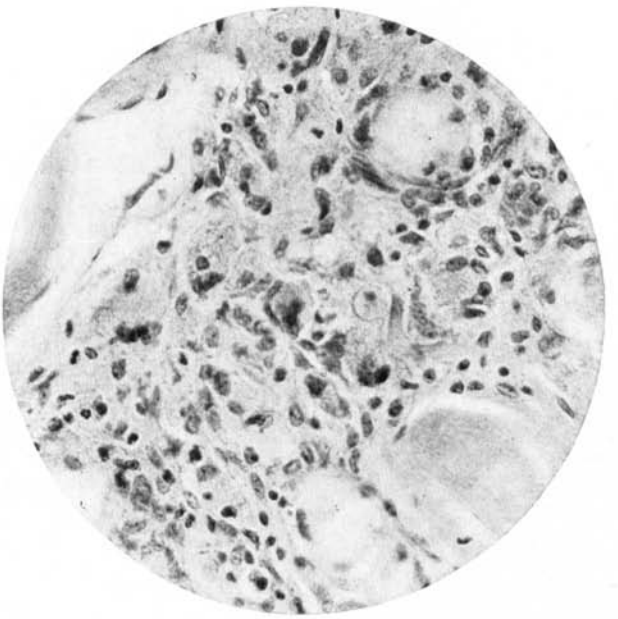

FIG. 26. Foamy granuloma cells. 300×.

FIG. 27. Marked vacuolation. 300×.

PLATE 26

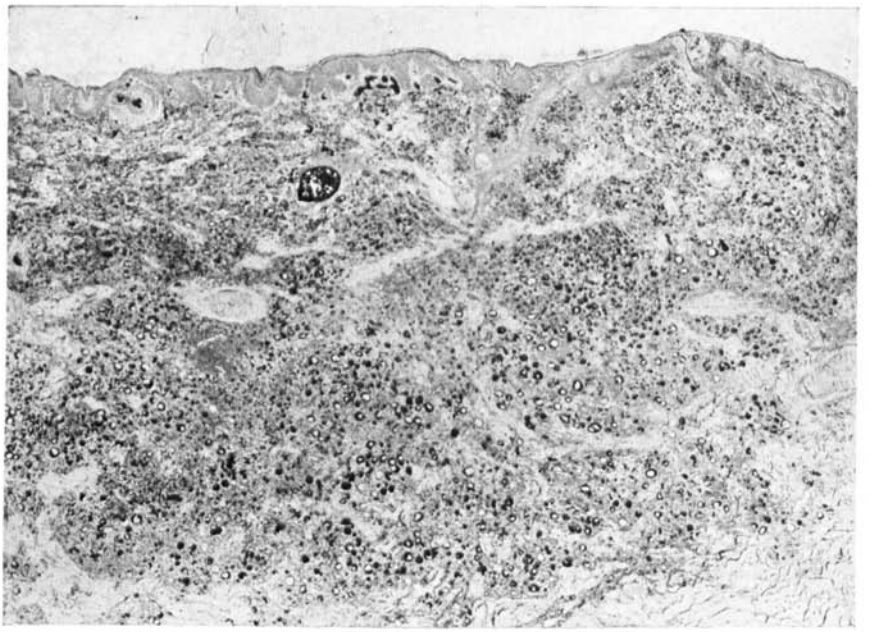

FIG 28. Globi in an infiltration, stained for bacilli. 22×.

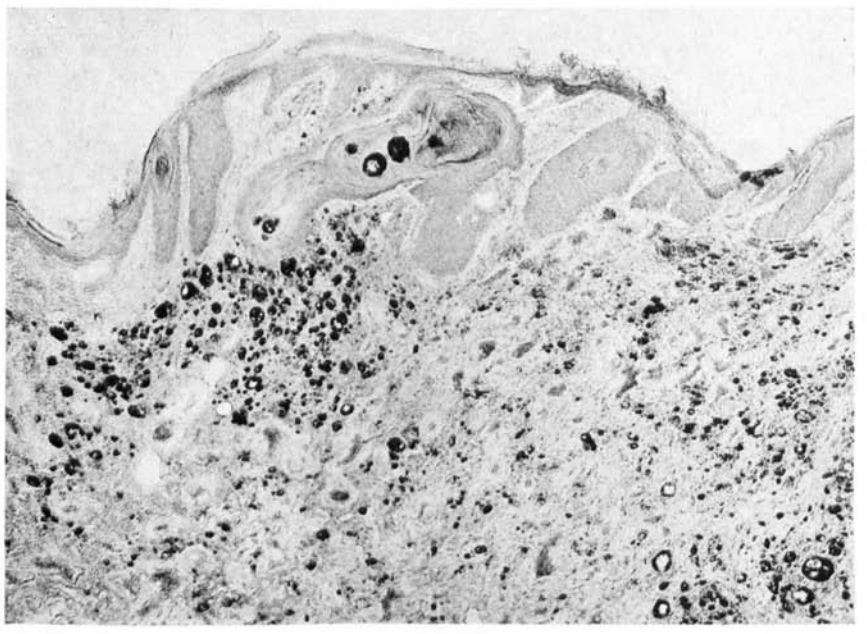

FIG. 29. Globi in a hair sheath. 65×.

PLATE 27

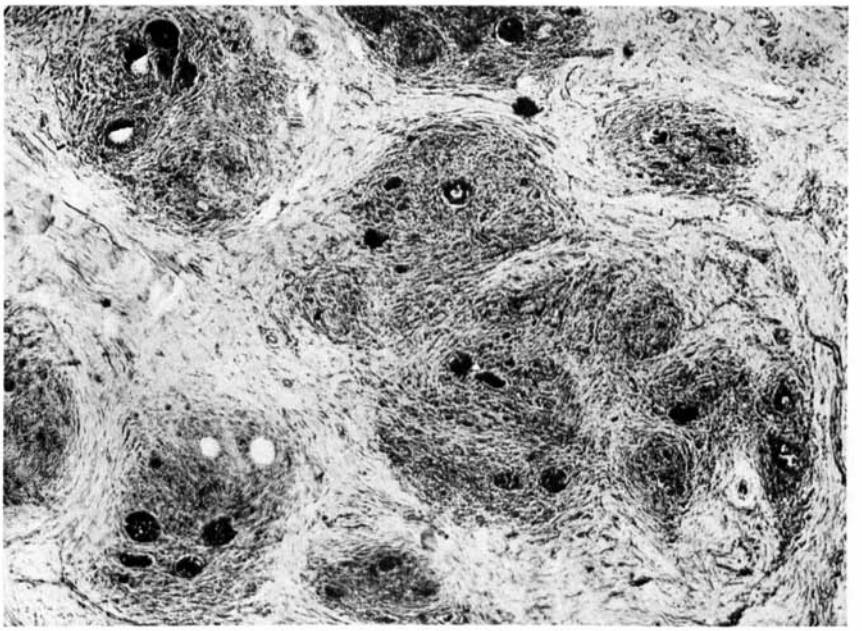

FIG. 30. Globi filling vacuole-like spaces. 65×.

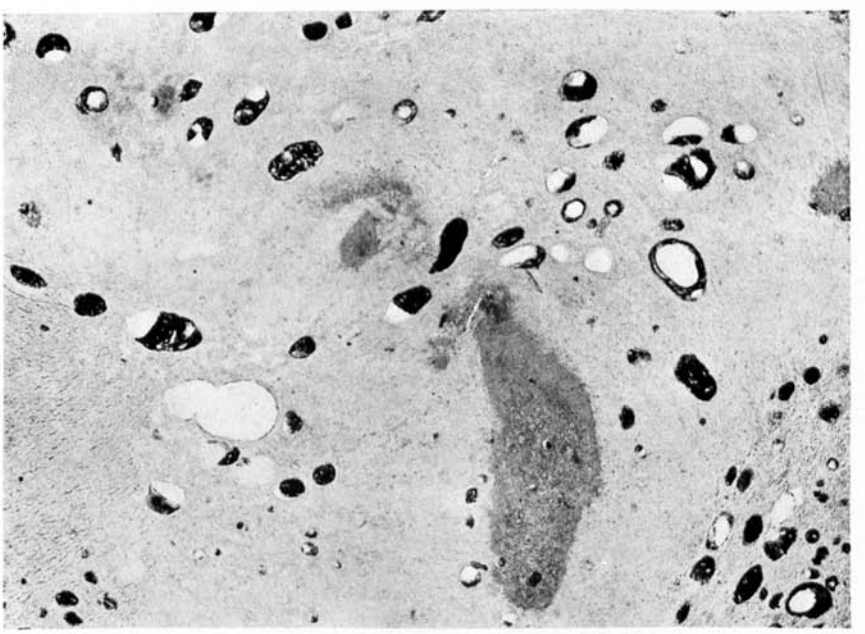

FIG. 31. Globi, with empty spaces, in large cavities. 65×.

PLATE 28

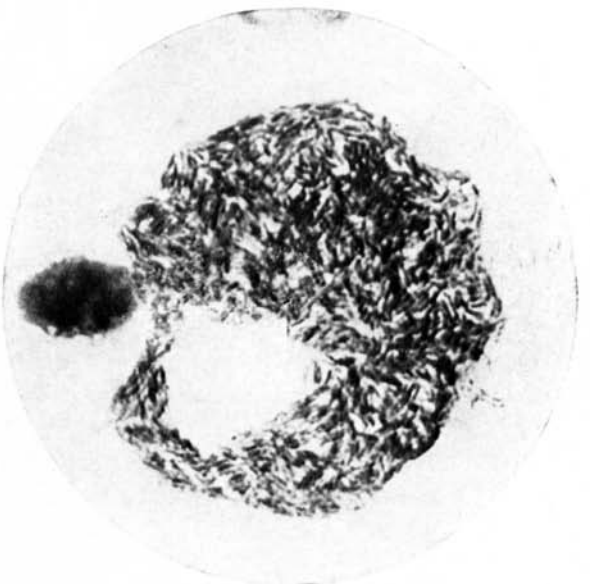

FIG. 32. Globus with empty space. Smear preparation. ±1400×.

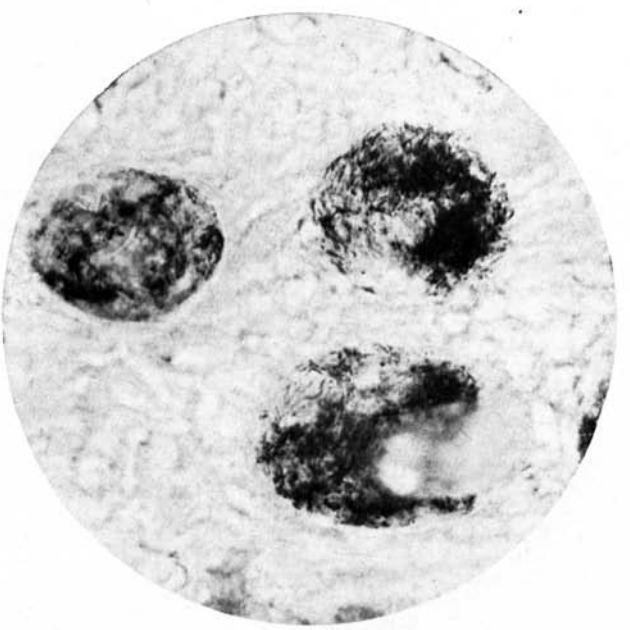

FIG. 33. Globi with rods and granules. 700×.

PLATE 29

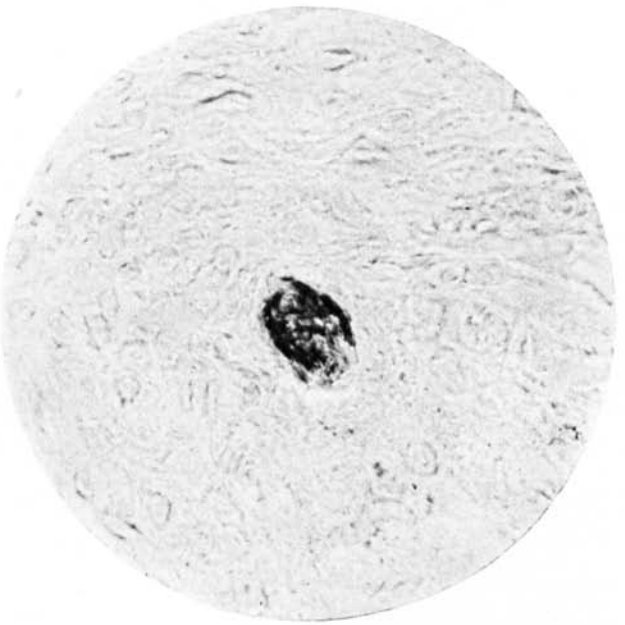

FIG. 34. Globus of solid rods embedded in fat. 700×.

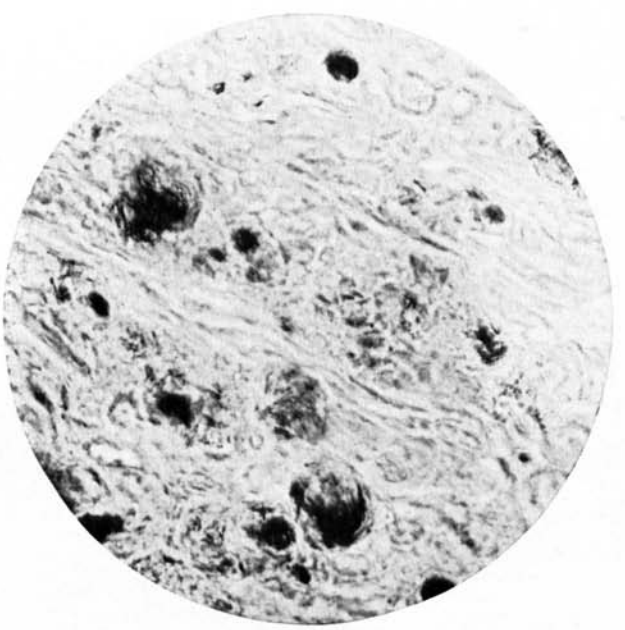

FIG. 35. Globi, bundles and dispersed bacilli. 700×.

PLATE 30

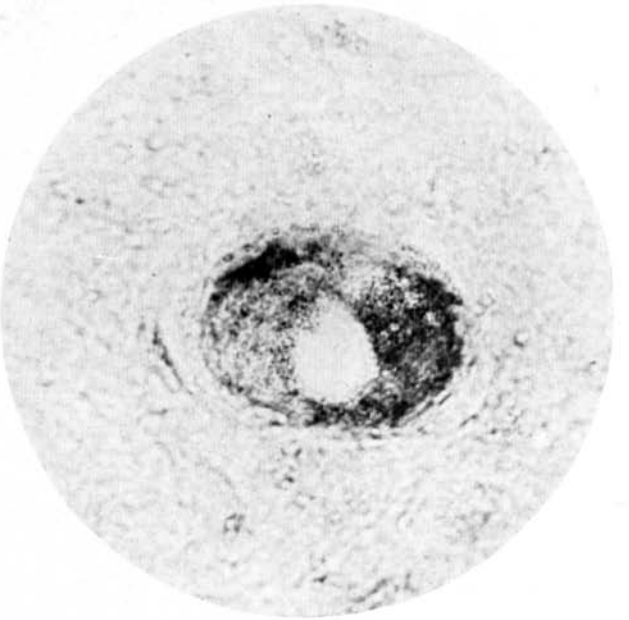

FIG. 36. Wreath-shaped globus of degeneration products. 700×.

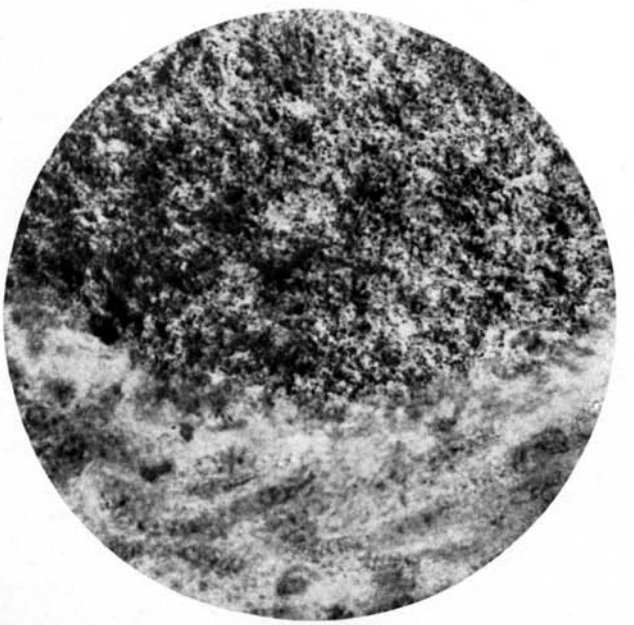

FIG. 37. Border of a very large bacillary cavity. 700×.

PLATE 31