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PURE GRANULOMATOUS NOCARDIOSIS: A NEW FUNGUS DISEASE DISTINGUISHED BY INTRACELLULAR PARASITISM

A DESCRIPTION OF A NEW DISEASE IN MAN DUE TO A HITHERTO UNDESCRIBED ORGANISM, NOCARDIA INTRACELLULARIS, N. SP., INCLUDING A STUDY OF THE BIOLOGIC AND PATHOGENIC PROPERTIES OF THIS SPECIES*

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Phagocytosis by reticulo-endothelial cells, together with proliferation of these cells, resulting from the presence of an offending agent, has been regarded generally as the fundamental basis of granulomatous inflammation. The majority of these reactions quickly become complicated by necrosis, fibrosis, alteration in structure of the macrophage, and appearance of other cellular elements such as lymphocytes and plasma cells. In human disease one rarely encounters a granulomatous reaction consisting entirely of phagocytosis and proliferation of macrophages. This is most nearly approached in the lesions of typhoid fever, histoplasmosis, and early stages of lymphogranuloma inguinale, but even here complicating features are to be found.

Opportunity was afforded recently to study a human reaction which assumed the form of phagocytosis with reticulo-endothelial cell proliferation and minimal necrosis. The agent which induced this reaction is an acid-fast organism which may be isolated easily in culture.

REPORT OF CASE

The patient was a white girl, 34 months old, whose illness began with anorexia, nausea, vomiting, and progressive weight loss about 4½ months before her death. Shortly after the onset a mass, thought to be a lymphosarcoma, was noted in the abdomen. The urine was reported as negative; hemoglobin, 8.4 gm. (52 per cent); red blood cells, 3,100,000; white blood cells, 20,150 (polymorphonuclear leukocytes, 33 per cent; eosinophils, 3 per cent; lymphocytes, 62 per cent; monocytes, 2 per cent). A transfusion of 225 cc. of type A blood and five treatments of deep x-ray therapy (dosage not known) were administered. Because of unfavorable reaction,

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radiotherapy was discontinued. She then was admitted to Duke Hospital for an exploratory operation.

Physical Examination. The child was emaciated; her skin was pale and dry. The lymph nodes of the axillary, cervical, and inguinal groups were enlarged. The chest was clear to percussion and auscultation; the heart showed no abnormality. Pulse was 120; blood pressure, 88/50 mm. Hg. In the abdomen, prominent in the upper quadrants, was a large, irregular mass. There was diminished muscular tone, especially of the extremities and abdomen.

Course. Shortly after admission an enlarged inguinal node was removed for study. The node showed complete alteration of its architecture with proliferation of the macrophages (Fig. 1). The cytoplasm of these macrophages appeared foamy and granular. Inasmuch as they resembled Gaucher's cells, a lipodystrophy was considered. However, by the use of Ziehl-Neelsen stain it was demonstrated that these cells contained massive numbers of an acid-fast bacilliform organism. A second biopsy showed a lesion of essentially the same type, and the organism was isolated in pure culture. Before the first biopsy was completed, the patient had received five x-ray treatments of 50 r., but upon the discovery of acid-fast organisms radiotherapy was discontinued. She then was treated by supportive therapy with intravenous supplemental proteins (amigen) and by chemotherapy, specifically, sodium p,-p'-diaminodiphenylsulfone-N,N' didextrose sulfonate (promin) and penicillin. The entire hospital course was afebrile except for two minor elevations of temperature to 38.5°C. and 38.8°C., respectively. The blood counts fluctuated: Hemoglobin, 8.1 gm.; red blood cells, 4.2 millions; white blood cells, between 7,500 and 19,360, with a differential formula of 26 per cent polymorphonuclear leukocytes, 38 per cent staff cells, 19 per cent juveniles, 4.5 per cent lymphocytes, and 12.5 per cent monocytes. Serologic tests for syphilis were negative. Total plasma proteins were 3.6 gm. per 100 cc.; albumin, 1.3 gm. per 100 cc.; globulin, 2.3 gm. per 100 cc.; albumin/globulin ratio, 0.56; cholesterol, 100 to 110. About 2 months before death the stools contained 30.49 per cent fat, with no parasites or ova; but many acid-fast organisms were found by both smears and culture. Other laboratory data were noncontributory.

On proctoscopic examination irregular ulcers were observed. A biopsy of one of these ulcers showed proliferation of macrophages containing many acid-fast organisms. Examinations of nasal smears for acid-fast organisms were negative. Intradermal tests with old tuberculin and avian tuberculin gave negative results, although the patient's serum was said to have agglutinated a suspension of her own organisms in a dilution of 1:640. The patient grew progressively weaker despite supportive measures, and died on her 78th hospital day, 4½ months after the onset of her illness.

AUTOPSY FINDINGS

Gross Examination

The body was emaciated, with accentuation of bony prominences. The *abdomen* was conspicuously distended. When the *abdominal cavity* was opened, approximately 500 cc. of chylous fluid escaped. Similar fluid was found in lesser quantity (150 cc.) in both *pleural cavities*. The *lacteal vessels* on the surface of the intestines were congested. The abdominal organs were displaced by a large, space-consuming mass in the mesentery and retroperitoneal regions (Fig. 2). This mass consisted of greatly enlarged and matted *lymph nodes*, which

on section were brilliant yellow. In the central portion of this mass were scattered, irregular zones of necrosis. The *pancreas, kidneys,* and *adrenals* were imbedded in the mass but not infiltrated by it, and were not otherwise abnormal. The *small intestine* showed no lesions, but ulcers occurred throughout the length of the *colon* (Fig. 3). These ulcers were eroded and indurated, with overhanging edges. Their bases were covered by blood-stained, greenish, necrotic material. The *lymph nodes* near the intestine were enlarged and bright yellow. The *spleen* was only slightly enlarged, and scattered throughout were yellowish areas which were interpreted grossly as malpighian corpuscles. The *liver* showed a prominent architecture with no focal lesions. The *mediastinal lymph nodes* were slightly enlarged. The *heart* and *lungs* were without gross lesions. The *brain* appeared normal. The *bone marrow* of the sternum, vertebra, and femur was pale, but gross lesions were not recognized.

Microscopic Examination

The architecture of the *spleen* was greatly distorted by an abundant proliferation of large, pale, foamy-appearing macrophages to the extent that lymphoid remnants appeared only as focal collections of cells (Fig. 4). The malpighian corpuscles were replaced by epithelioid cells, concentrically arranged about the central artery (Fig. 8). These structures were delineated by what appeared to be collapsed sinusoids containing small quantities of blood. Lymphocytes were virtually absent, although a few scattered plasma cells could be found. There was no conglomeration of these structures, despite their juxtaposition. The epithelioid cells contained numerous intracellular acid-fast organisms (Fig. 10), simulating the parasitism found in leprosy and Johne's disease. There were a few isolated organisms of varying lengths in the interstices. Branching forms were not observed. Groups of multinucleated giant cells, usually in clusters of a dozen or more, occurred throughout the spleen. The nuclei were in a peripheral position, and, for the most part, were small and hyperchromatic; only a few of the pale, vesicular type were found. In the central zone of the cytoplasm there was a clear, spherical, homogeneous area (Figs. 9 and 11) which proved to be devoid of organisms, but the foamy, granular cytoplasm around it contained acid-fast organisms, usually radially arranged. Foot's silver preparations failed to demonstrate reticulum, and even with Masson's trichrome stain little fibrosis was recognized.

The architectural pattern of the *lymph nodes* was as distorted as that of the spleen (Fig. 5), and there were similar groups of large giant cells

(Figs. 8 and 11). The epithelioid pattern found in the spleen was nowhere apparent in the nodes. In some zones the multinucleated giant cells were so numerous as to form broad sheets. Isolated areas of necrosis were found in the retro-aortic and mesenteric lymph nodes. Only a few of these areas were large. Elsewhere necrosis was not a feature of the lesion. Several recanalized thrombi in fairly large arteries were noted.

The floor of the *ulcers in the colon* consisted of fibrous scar tissue, with intermingled groups of macrophages containing very numerous acid-fast organisms. There were remarkably few leukocytes and plasma cells, commonly seen in intestinal ulcers. Groups of macrophages with phagocytized organisms were noted also in nonulcerated areas, interpreted as replacement of lymphoid follicles by reticulo-endothelial cells. They frequently interrupted, by infiltration, the muscularis mucosae. The ulcers were thought to arise by surface erosion of the masses of proliferating macrophages.

Scattered tubercle-like nodules of epithelioid cells occurred in the *liver* (Fig. 6). The epithelioid cells contained many acid-fast organisms (Fig. 7). Many Kupffer cells throughout the liver showed phagocytosis of the organism, but there was no apparent damage to liver cells. In routine preparations of the *lung* no lesions were found. However, by acid-fast technics, organisms were demonstrated in the submucosal lymphoid follicles of the bronchi and in perivascular macrophages. Here again, massive intracellular parasitism was apparent.

In the mucosa of the *appendix* the lymphoid element was somewhat proliferative, and here also were many intracellular acid-fast organisms; but no tuberculoid arrangement was seen. The tissue about the *kidneys, pancreas,* and *adrenals* contained foci of epithelioid proliferation and there was intracellular parasitism. However, lesions were not found within the parenchyma save for scattered calcium deposits in the medullary tubules of the kidney.

In sections of the *myocardium,* calcific subendocardial deposits were found in the vicinity of the mitral valve, and there was a mild vacuolization of myocardial fibers, but no granuloma.

Microscopic studies of the *bladder, uterus, ovary, diaphragm, thyroid, pituitary body,* and *brain* were noncontributory. These organs appeared normal; no aggregates of organisms were found.

Anatomic Diagnoses. A peculiar form of granuloma characterized by intracellular parasitism and proliferation of macrophages producing complete replacement of lymphoid tissue of mesenteric, retroperitoneal, mediastinal, and left subclavian lymph nodes, and spleen; partial re-

placement of bone marrow and lymphoid tissue of gastro-intestinal tract; and microscopic proliferation of macrophages with intracellular organisms in liver and lungs; granulomatous ulceration of colon; congestion of lacteal lymphatics, chylous ascites (500 cc.), bilateral pleural effusion (150 cc.); calcium deposition in subendocardial tissue and renal tubules; and emaciation.

INTERPRETATION

The outstanding feature of the disease in this case was the peculiar host-parasite relationship. The sole local reaction appeared to be a stimulation of phagocytic activity of the macrophages of the reticulo-endothelial system with proliferation of these phagocytes. As a consequence, the predominant lesions occurred in the spleen, lymph nodes, and bone marrow. This feature was emphasized further by the fact that in the small lymphoid collections of the bronchi, in the liver, and around the vessels in the pancreas was found marked phagocytosis of these organisms by large macrophages. This process could not be appreciated in ordinary preparations and was recognized only when the tissue was stained by the acid-fast technic.

This host-parasite relationship was characterized further by the predominantly intracellular position of the parasite. However, in a few areas, particularly in the lymph nodes and the spleen, extracellular organisms were noted; but with the impressively dominant intracellular parasitism organisms in the interstices were interpreted as having "spilled over" from the macrophages. The organisms appeared to be in equilibrium with the cells and thus were capable of growth and multiplication. The formation of giant cells, with peripherally placed nuclei and a clear central zone, was interpreted as support for the concept of Doan, Sabin, and Forkner [1] regarding the formation of giant cells of the Langhans' type by amitotic division of macrophages. By supravital staining these authors have shown that the peripheral disposition of the nuclei in Langhans' giant cells is due to the centrally placed "rosettes of fine vacuoles," which in the living state are stainable with neutral red. It seems likely, therefore, that the clear central zones seen in many of the giant cells in the lymph nodes and in the spleen of this case were due to the presence of such "rosettes."

This host-parasite relationship seemed to be characterized further by the fact that no product of metabolism of the organism was toxic to its host. Such a point of view seems substantiated by the fact that throughout her illness the patient did not exhibit a consistent febrile response. In addition, there was no leukotaxis, in that none of the

lesions showed significant numbers of polymorphonuclear leukocytes or other elements of suppurative inflammation. In this connection it is noteworthy that early in her illness there was an absolute lymphocytosis. However, following x-ray therapy and progress of the disease a degenerative shift of the hemogram occurred. Necrosis was not a conspicuous feature. There were zones of necrosis in the large masses of lymph nodes of the mesenteric and retroperitoneal groups, but the more discrete nodes and the spleen did not show this feature. It may be pointed out that the large mass in the abdomen had received a total dosage of approximately 500 r. of deep x-ray therapy. In addition, two large vessels with old recanalized thrombi were found in these nodes at autopsy.

Intracellular parasitism by an acid-fast organism, as presented here, finds its analogue in human leprosy, Johne's disease in cattle, and rat leprosy. However, in human leprosy there are elements in the process of inflammation other than those which characterize the disease under consideration, and the distribution of lesions is different. Furthermore, the organism of leprosy is recovered with extreme difficulty, if it can be cultured at all. Rat leprosy of the glandular type presents a lesion which is strikingly similar to that found in our case. On comparing sections of rat leprosy obtained through the courtesy of Dr. R. D. Lillie of the National Institute of Health, we have found that, while there is extensive phagocytosis and macrophagic proliferation, the lymph node structure does not show the widespread alteration that is the rule in our case. The organism of rat leprosy is more difficult to stain and has greater variation in structure than the organism in our case. Moreover, the organism of rat leprosy has not been successfully cultured by ordinary methods.[2]

In Johne's disease there are reacting elements other than the reticuloendothelial cells. The lesions are distributed chiefly throughout the gastro-intestinal tract, and the organism possesses cultural and morphologic characteristics which differentiate it sharply from that responsible for the disease in our case.

A consideration of the mechanism of death in this patient suggests a number of interesting possibilities. The patient obviously was suffering from severe inanition. While it is evident that absorption of nourishment from the gastro-intestinal tract was impaired, it does not seem likely that this factor alone could account for the severe metabolic disturbance. Moreover, the analogy of the situation to that of advanced malignant disease is sharply apparent. Perhaps the same undetermined factors which produce death in certain cases of cancer were operative

in this case. This view finds some support in the extraordinary tumor-like proliferative activity of the reticulo-endothelial cells which constituted a typical feature.

Nature of the Etiologic Agent

Isolation

Isolates of this organism were obtained first from a retroperitoneal lymph node removed for biopsy, later by culture of stools, and finally from the spleen and a lymph node at autopsy. Small acid-fast organisms were seen in smears made from the ileum, jejunum, colon, spleen, abdominal lymph nodes, urine, and ascitic fluid. They did not appear in direct smears from the heart's blood, lungs, liver, kidney, and spinal fluid, although they were subsequently stained in sections of liver and lungs. Post-mortem cultures were freed from contaminants by digestion with normal sodium hydroxide for 30 minutes. Observations were made on isolates obtained both before and after the death of the patient.

Cultivation

Gross Morphologic Features on Solid Media. On glycerin egg medium, growth appeared in 24 to 28 hours as smooth, minute, shiny, pale yellow colonies which rapidly increased in size and pigmentation. After 3 to 7 days the colonies became confluent, definitely elevated, shiny, pale yellow to yellowish orange, and butyrous (Fig. 12). A sweet, yeast-like odor was produced irrespective of the substrate used. Aerial mycelium did not appear at any stage of cultivation, and there was no discoloration of the media. Growth on plated media, while not unlike that on slants, was somewhat slower. On Sabouraud's agar, growth was visible in 36 to 48 hours, and resembled the young colonies produced on glycerin egg slants. As growth increased, however, the color became gray to grayish yellow. With ageing the colonies assumed the characteristic deeper yellow to yellowish orange. The growth eventually became more elevated than on glycerin egg medium, and vermiform (Fig. 13). On plates of sheep's blood-agar, growth was slow, and a slight, irregular hemolysis was produced. Czapek's agar plates or slants with carbohydrate did not support abundant or characteristic growth, although subcultures grew rapidly and well when transferred from this medium to glycerin egg substrate. On Bordet-Gengou agar, colonial development was similar to that obtained on glycerin egg and Sabouraud's media. On Löffler's serum slants, pigmentation of the colonies was increased, so that a deep yellow-orange color was produced. Growth on the Löffler slants was peculiar in that it apparently terminated after

3 to 4 days. Subcultures from this to other media were normal in colony characteristics.

Growth in Liquid Media. Beef extract broth plus 5 per cent glycerin favorably supported growth. In 3 to 7 days a web-like mucoid clot was formed, primarily in the butt of the tube; however, there might be some extension of the growth over the dependent side of the tube. The broth remained clear and there was no pellicle. Prolonged incubation (30 to 40 days) resulted in a slight, increasing turbidity, the degree of which depended upon the amount of inoculum used. The broth was not discolored. In beef extract broth without glycerin and in beef infusion broth without glycerin there was no visible growth after 6 to 7 days. Beef extract broth containing 5 per cent glycerin and 0.5 per cent phenol supported growth which resembled the cultures in glycerinated beef extract broth. Transfers from phenolized broth grew rapidly and well, with no change in gross cultural characteristics. The growth in Sabouraud's broth was similar to that in beef extract broth with glycerin. Proskauer-Beck broth containing asparagin and Dubos' (Tween) broth supported growth, but not as well as did beef broth containing glycerin or Sabouraud's broth.

Viability, and Oxygen and Temperature Requirements. Cultures remained viable for months on slants of glycerin egg medium, and successful transplants were obtained from cultures 1 year old. A culture, with or without other organisms present, resisted digestion by N sodium hydroxide for a period of 30 minutes. The organism was aerobic, but grew in a candle jar containing approximately 10 per cent CO_2. There was no growth in the Brewer anaerobe jar and the organism died within 14 days. The optimum temperature for growth was 37.5°C., but there was good growth at room temperatures. No growth occurred at 40°C., and the organism was killed at 60°C. in 10 minutes.

Biochemical Reactions

In fermentation broth to which 1 per cent xylose or 1 per cent arabinose had been added, acid without gas was produced in 5 to 10 days. No fermentation occurred in broths containing 1 per cent of lactose, dextrose, sucrose, mannite, trehalose, or sorbitol. In litmus milk there was a slight acidity within 20 to 30 days. Peptonization did not occur and growth seemed to be more abundant in the lower portion of the tube. After incubation for 35 days in gelatin, there was neither visible growth nor liquefaction. Indole was not formed, nor was tyrosinase produced, since there was no brownish discoloration of the peptone broth. Nitrates were reduced to nitrites. Cellulose was not decomposed in Dubos' cellulose-nitrate broth, although this medium supported a

slight growth. In potato-dextrose-starch agar little or no growth occurred, and there was no diastasis of the starch.

To determine the utilization of paraffin as the sole source of carbon, a glass rod coated with sterile paraffin was suspended in a flask of carbohydrate-free Czapek's broth. Previous to the insertion of the paraffin-coated rod a 0.2 cc. saline suspension of the organism had been added to the medium. After 7 days' incubation at 37.5°C., the paraffin-covered rod was coated with a dust-like, yellow film. The rod was streaked across the surface of glycerin-egg medium slants, and typical growth of the organism occurred in 4 to 7 days. A stain of a smear from the rod showed many acid-fast organisms.

Microscopic Morphology and Staining Reactions

The organism was pleomorphic, Gram-positive, acid-fast, non-spore-forming, and when undisturbed exhibited true lateral branching. In smears of tissues and exudates from the patient and from experimental animals intracellular bacillary forms predominated, but some free bacillary and a few coccobacillary forms were present. Some of the bacillary forms contained large pleomorphic granules, and all free organisms tended to assume a diphtheroid-like arrangement. There were no ray formations in exudate. In all preparations the organism was 0.2 to 0.45 μ in diameter. The longer elements contained small spherical to ovoid bodies at regular intervals throughout their lengths. These ovoid structures were extremely acid-resistant and Gram-positive. In addition, large pleomorphic structures were found, at times terminally and sometimes irregularly placed throughout the organisms. These were wider than the remainder of the organism. They were not acid-fast, but took the counterstain intensely and were Gram-positive. It may be pointed out that in fat-enriched and glycerinated media the number of these bodies was increased.

On Sabouraud's agar in Van Tieghem cell mounts the organism consistently produced branching filaments in 5 to 8 days. These branches formed a densely matted mycelium in the central portion of the mount. Delicate, tangled, branching hyphae, 0.2 to 0.45 μ in width, were formed at the periphery of the growth (Fig. 16). There was no indication of dichotomous branching, chlamydospores, or chains of spores such as may be seen in those aerobic actinomycetes which produce an aerial mycelium. In cultures other than the Van Tieghem single cell mounts, branching was inconspicuous when examined in smears made by loop transfers (Fig. 15). However, in litmus milk long branching forms were prevalent, and large tangled masses were commonly observed (Figs. 17 and 18). Because of the consistent branching and

the growth on Sabouraud's medium at room temperature, the organism is regarded as a fungus. The small, round to ovoid, regularly placed bodies, therefore, may be interpreted as arthrospores; and the irregularly spaced, pleomorphic, non-acid-fast structures may be termed granules. Therefore, the organism appears structurally as a series of arthrospores bound together by a thin membranous sheath.

Throughout the study of this organism its consistent and extraordinary resistance to decolorization with acid or acid-alcohol was an impressive feature. In smears made from exudate and from freshly isolated cultures the organism was partially decolorized by 25 per cent hydrochloric acid-alcohol* in 1 hour. However, preparations made from litmus milk cultures remained completely acid-fast in 25 per cent hydrochloric acid-alcohol for over 60 minutes. On the other hand, the acid resistance was so diminished by culturing on Sabouraud's medium that the organism was totally decolorized by 2 per cent hydrochloric acid-alcohol in 3 minutes. Acid-fastness was influenced in part by the hydrogen ion concentration of the medium. Increase in either alkalinity or acidity of the medium resulted in a correlated diminution of acid-fastness. The greatest resistance to decolorization was obtained by cultivation at a pH range of 7.2 to 7.6. The age of the culture, however, had no influence, for a 16-months-old culture on glycerin egg medium retained its acid-fastness to a degree comparable to that of fresh cultures.

Regardless of the medium used, the relative acid-fastness of the component parts of the organism was constant. The arthrospores were the most vividly stained by the fuchsin; in contrast the rest of the hypha, although clearly acid-fast, was pale. The granules were never acid-fast.

Analysis of Characteristics

The outstanding features of this organism are its high degree of acid-fastness, its consistent branching on media permitting direct observation, and its resistance to sodium hydroxide digestion. The acid-fastness rivals that found in the mycobacteria and exceeds that usually encountered in the nocardia. However, the acid-fastness of species of Nocardia varies considerably; a few strains have been described as "strongly acid-fast" [3] and "as resistant to acids as the tubercle bacillus." [4] The constant branching of the organism under investigation and its fragility as observed when transferred by loop to smears are suggestive of the aerobic strains of actinomycetes for which Waksman and Henrici [5] employed the generic name Nocardia. It is well known that branching forms of mycobacteria exist. However, they are found

* Prepared by using 25 cc. of C.P. HCl plus 75 cc. of 95 per cent alcohol.

only rarely and under unusual conditions, as in smears from old cultures and sometimes in exudates.[6] This feature of mycobacteria is not consistent even in the same strain. The resistance of our organism to sodium hydroxide digestion is difficult to evaluate as a criterion of classification. This procedure is generally employed to free exudates of organisms other than *Mycobacterium tuberculosis*. However, it may be that the factors responsible for resistance to acid decolorization on the part of an organism may also protect it against sodium hydroxide digestion.

Comparison with Other Organisms

Mycobacterium tuberculosis. As indicated above, the organism here described has a number of morphologic features in common with *M. tuberculosis*. The similarity of fragments of the hyphae to tubercle bacilli is striking, especially when the two are compared in photographs made with the electron microscope. However, the resemblance of our organism to *M. tuberculosis* goes no further than this. The consistency of its branching, its ability to grow on a multitude of media at extremely variable temperatures and hydrogen-ion concentrations, its ability to utilize paraffin as its sole source of carbon, its unique relationship to the cell of its human host, and its failure to kill experimental animals differentiate it sharply from any known form of virulent *M. tuberculosis*.

Other Acid-Fast Bacteria. Intracellular parasitism and acid-fastness, both important characteristics of our organism, are also characteristic of *M. leprae*. Beyond this, however, resemblance between the two completely disappears. The ease with which growth of our organism is obtained would seem to eliminate any possibility of confusing it with *M. leprae* in view of the well known difficulty and special technics required in culturing the leprosy bacillus.[7-9] For example, the organism isolated by Clegg [8] from a case of leprosy is only weakly acid-fast and extremely fastidious in its growth requirements on primary isolation, features which distinguish it sharply from our organism. The organism from Duval's [9] case of leprosy, unlike our organism, required 1 to 7 months on highly enriched media for primary isolation, and the colonies produced were glistening and white. A number of acid-fast bacilli have been isolated from cases of leprosy by other workers, but absolute proof that these organisms are causative of this disease is lacking.[10] From the studies of our own organism in its relation to the disease in the host in which it is found, and in relation to experimentally produced disease in a variety of animals, as described in a subsequent section, there is no question of its etiologic specificity.

Johne's disease of cattle and rat leprosy show intracellular parasitism by acid-fast organisms comparable to that in our case. The organism

of rat leprosy, like that of human leprosy, has not been cultured by ordinary methods,[2] and the organism of Johne's disease differs from our organism in the following ways: Johne's bacillus requires for its growth the addition of extracts of other acid-fast organisms to the primary isolation medium; it requires 4 weeks for growth to become apparent; dull yellowish white, striated colonies are produced; maintenance requires enriched media, and branching has not been observed.[11]

The remaining acid-fast organisms, considered saprophytic mycobacteria, offer problems of comparison with our organism which cannot be solved adequately. For example, statements regarding their ability to branch on Van Tieghem cell mounts are not included in their descriptions. Through the courtesy of Dr. William A. Hagan of Cornell University we have been able to compare our organism with some of these acid-fast bacteria, including one strain each of *M. phlei, M. ranae,* and *M. leprae* (the Clegg II bacillus), and five acid-fast soil saprophytes. For the same purpose, Dr. Hagan also supplied us with three acid-fast organisms isolated from cattle, and one isolated from a case of human leprosy, labeled Kat no. 352. To this collection was added a strain of *M. graminis* obtained through the courtesy of Dr. W. Steenken of the Trudeau Sanatorium. All of these organisms were propagated on Van Tieghem cell mounts, Sabouraud's agar, and glycerin egg medium, and in litmus milk. The organisms were studied both at room temperature and at 37.5°C. Branching, a highly significant characteristic of our organism, was not observed on the cell mounts in the case of *M. graminis, M. phlei, M. ranae,* the Clegg II bacillus, the cattle isolates, and soil saprophyte no. 135; nor was true branching observed in smears made from litmus milk cultures of these organisms. On the other hand, four of the saprophytic soil strains designated by Dr. Hagan as "without identification" [12] and the Kat no. 352 organism showed abundant and true branching. These branching organisms differ from our organism, however, as follows. Kat no. 352 organism and soil saprophyte no. 121 do not grow on Sabouraud's medium. In addition, the Kat no. 352 strain will grow at 47°C., while the organism considered here resists cultivation at 40°C. and beyond. Soil saprophytes nos. 136 and 127 differ from our organism in that they form a pellicle in broth and produce dry, rough colonies on solid media. Soil saprophyte no. 132 differs from our organism in its scant growth on Sabouraud's agar and its dry, flaky colonies. Further studies of these organisms are in progress. However, since they too are unclassified, identification of the organism here reported with one of these, even though unlikely, would be of no assistance in its ultimate classification.

Actinomycetes. The majority of the organisms belonging to the

family Actinomycetaceae may be eliminated from consideration in connection with the identification of our organism because they are not acid-fast. Nevertheless, there is a considerable group of aerobic, slightly acid-fast actinomycetes which grow in the form of branched, vegetative hyphae. These hyphae readily undergo fragmentation and thus give rise to bacillary and coccoid arthrospores. It is to this group of organisms that the generic name Nocardia is given. Since the organism under discussion seems most closely allied to this group, the several species of Nocardia will be discussed in some detail.

In 1888, Nocard [13] described an aerobic, acid-fast, filamentous organism isolated from farcy of cattle which was identified by Gasperini [14] as *Actinomyces farcinicus*. In 1890, Eppinger [15] described *Cladothrix asteroides,* another aerobic acid-fast actinomycete, as the cause of an infection of the brain and meninges in man. This organism was renamed *Nocardia asteroides* and has been designated as the key species of the genus, Nocardia. In 1921, Henrici and Gardner [16] collected reports of 26 instances of human infection by a variety of strains of aerobic acid-fast actinomycetes, Nocardia, and were able to group the organisms into three distinct species. The first of these is *N. asteroides*. This organism is characterized by a mealy growth on agar with pale yellow to deep orange colonies; it does not liquefy gelatin or peptonize milk; it is more highly pathogenic for guinea-pigs than for rabbits. The second species is typified by a strain isolated by Birt and Leishman [17] and later named *N. leishmani* by Chalmers and Christopherson.[18] This organism produces a snow-white growth on solid agar, peptonizes milk, and does not liquefy gelatin. It is pathogenic for guinea-pigs and is reported to be pathogenic for rats and rabbits. The third species is that described by Berestneff.[19] It forms a gray to whitish colonial growth on solid media, liquefies gelatin, and is not pathogenic for laboratory animals. To these three species Henrici and Gardner [16] added a fourth, *A. gypsoides*. Henrici [20] later declared this to be not unlike *N. asteroides*.

In addition to the above-described species, five other species of acid-fast actinomycetes which were recovered from infections in man were found to be described in the literature. From a case of pulmonary actinomycosis, Davis,[21] in 1914, observed such an organism in the sputum of a 64-year-old-man, which he described as an "acid-fast streptothrix." He was not able to culture this organism, nor to determine its pathogenicity for animals. In 1920, Vuillemin [22] isolated an acid-fast, Gram-negative actinomycete from a case of bubonic plague and called it *N. jollyi*. Davis and Garcia,[23] in 1923, isolated an acid-fast organism from subcutaneous abscesses on the extremities of a woman. They assigned their organism to the genus Nocardia, but did not iden-

tify it fully. Later a group of investigators [24] considered this organism akin to *N. asteroides*. In 1934, Gammel,[25] assisted by Werkman, isolated a new species of acid-fast actinomycete from a human infection and, because of its ability to grow in phenolized media, called it *A. phenotolerans*. In 1937 Goldsworthy [26] described an acid-fast actinomycete which was later considered by Kirby and McNaught [27] to be a variant of *N. asteroides*.

The taxonomic distinction between the acid-fast nocardia and the mycobacteria is difficult because of characteristics held in common by organisms of these two groups. This difficulty was acknowledged by Umbreit [28] in his classification, wherein he set up special groups which he called proactinomycetes. He divided this group into the alpha proactinomycetes which are closely related to the mycobacteria and corynebacteria because of their unstable mycelium in slide cultures, and the beta proactinomycetes which are allied to the true actinomycetes because of their stable mycelium and "actinomycete growth in liquid media." The alpha and beta proactinomycetes are further divided into those which are not acid-fast and are therefore akin to the corynebacteria, and those which are acid-fast and are therefore like the mycobacteria. The organism of this present communication can then be classified as one of the acid-fast beta proactinomycetes.

Rosebury [29] classified the actinomycetes according to their parasitic or saprophytic properties, with a species differentiation based on morphologic, environmental, and cultural characteristics. The organism of this report does not lend itself to identification with one of these groups because it possesses both the properties ascribed by Rosebury to the parasitic type and those belonging to the saprophytic types.

The classification of the actinomycetes most useful to us and, we believe, generally acceptable is that of Waksman and Henrici [5] (1943), which is based on the character of the branching of these organisms. In their classification, the family Actinomycetaceae is divided into aerobic and anaerobic genera. The aerobic groups of organisms, partially acid-fast or non-acid-fast, which form a vegetative mycelium dividing by segmentation into bacillary or coccoid elements (arthrospores [30]) but do not produce conidia, are designated Nocardia.

Taxonomic Position of the Etiologic Agent

The placement of the organism dealt with in this report offers difficulty. That it belongs in one of the above-discussed groups of organisms seems obvious because of its acid-fastness. That it is not *M. leprae*, the organism of Johne's disease, or the organism of rat leprosy is indicated

by the ease with which it is cultivated. The degree of its acid-fastness would tend to place it among the mycobacteria; however, the consistency of its branching and its fragmentation into bacillary and coccoid forms would seem to place it among the nocardia. In addition, its ability to grow on Sabouraud's medium at room temperature would appear to confirm the fungous character of this organism. The conclusion is drawn, therefore, that this organism should be more properly considered a species of Nocardia.

In contrasting this organism with the several species of Nocardia, as outlined by Henrici and Gardner,[16] sharp differences become apparent. It differs from *N. asteroides,* the type species of this genus, in that it shows a greater degree of acid-fastness, it resists digestion by sodium hydroxide, it produces buttery colonies, it does not produce aerial mycelia, it does not develop other than yellow pigment, it does not form a pellicle in liquid media, it grows poorly on potato agar and gelatin media, and it fails to produce a lesion in rabbits. It differs from *N. leishmani* in that it does not produce a snow-white growth on agar (that is, it forms no aerial mycelia); it does not produce peptonization of milk, and it is not pathogenic for rabbits. It differs from the species reported by Berestneff [19] because it does not produce gray to white growth on solid media and is pathogenic for laboratory animals. It differs from *A. gypsoides* because it does not produce white colonies, it has no proteolytic activity, it does not produce tyrosinase, and is not lethal to laboratory animals. Equally sharp differences between our organism and those strains which are not included in the classification of Henrici and Gardner are apparent. Since the acid-fast streptothrix described by Davis [21] could not be cultured, and its pathogenicity could not be determined, its differentiation from our organism seems obvious. *N. jollyi,* reported by Vuillemin,[22] proved to be Gram-negative, which differentiates it from the organism here reported. *A. phenotolerans,* of Gammel and Werkman,[25] is similar to our organism in that it was extremely acid-fast and capable of growing in a medium to which was added 0.5 per cent phenol, but it differs in that it formed a pellicle in liquid media and produced aerial mycelia.

Summary

Inasmuch as this organism is considered a species of Nocardia, and inasmuch as there are sharp differences between it and known species of this genus, it is concluded that this organism represents a hitherto undescribed species; for this new species the name *Nocardia intracellularis, n. sp.,* is suggested because of the characteristic intracellular

position which it occupies in the human host. A brief characterization in Latin with English translation is as follows:

Nocardia intracellularis, sp. nov. Filamentis ramosis, in fragmenta secendentibus, ex baculis seriatis constitutis, 0.2-0.45 μ latis; in culturis in liquoribus colonias ramosas efformat; apice non clavatis; chlamydosporis non efformantibus; arthrosporis ellipsoideis apice efformantibus; non-motilibus. Hyphae colorantur per fuchsinam acidospiritu-rectificato haud decolorantur. Difficulter propagatur in gelatina, atque gelatinam non liquefacit. In culturis agar-agar applantis colonias circulares, elevatas, nitentes, leves, non mucosas, post 3-6 dies.

Fluidium hyalinium in culturis liquidis glycerino atque massas, albas, mucosas, ad basem tubuli efformantis. Lac coloratum cum litmo, acidum fit post 20-30 dies. In culturis Sabouraudii vegetat rapide post 3-7 dies. Amylum non vertit. Decompositionem cellulosae non provocat. Tyrosinase non efficit. Indole non efficit. Decompositionem potassae nitrasi non provocat. Non viget in infusionibus tuberorum solani agar-agar. Crescit in jure carnis in quo glycerino atque 0.5 per centum acidum carbolicum adest. In oxygenii absentia non evolvitur sed in 10 per centum carbonei dioxidi viget. Cera origine sola carbonis utor potest. Viget rapide ad temperaturam 37.5°C. Facile evolvitur usque ad temperaturam 40°C.

Habitat: In granulomis organorum infectorum lymphaticorum observatus est, ac in faecibus homini vivi in quo morbum causart, atque in cadavero in laesionibus granulomatis disseminatis late quas edit.

Nocardia intracellularis, n. sp. Filaments branched, becoming fragmented, composed of bacillary elements in series, 0.2 to 0.45 μ in width; in cultures in liquids, branched colonies are formed; not club-shaped at the tips; and lacking chlamydospores; nonmotile. The hyphae are not at all discolored when stained with fuchsin and treated with acid alcohol. It grows poorly in gelatin, which it does not liquefy. In agar plate cultures the colonies are circular, raised, wet-shining, smooth, and non-mucoid after 3 to 6 days.

In glycerin broth the fluid remains clear; and white, mucoid masses are formed at the bottom of the tube. Litmus milk becomes acid after 20 to 30 days. On Sabouraud's agar it grows well after 3 to 7 days. Starch is not changed. It does not decompose cellulose. Tyrosinase is absent, and indole is not produced. It does not decompose nitrates of potassium. It does not thrive on potato agar. It grows in beef extract broth to which has been added glycerin and 0.5 per cent carbolic acid. It does not develop in the absence of oxygen, but grows in an atmosphere having 10 per cent CO_2. It can use paraffin as a sole source of carbon. It thrives at 37.5°C. and tolerates well temperatures up to 40°C.

Habitat: Observed in granuloma of infected lymph nodes and in the feces of a living patient whose death it caused. Observed also at autopsy in widely disseminated granulomatous lesions which it produced.

PATHOGENICITY OF NOCARDIA INTRACELLULARIS

The study of the reaction of animals to inoculation by *Nocardia intracellularis* was divided into three parts: The determination of species susceptibility; a study of the route of infection; and a study of the development of the lesion.

Species Susceptibility

In the experiments designed to determine which animals would become infected, chickens, rabbits, goldfish, frogs, rats, mice, and guinea-pigs were used (Table I). The standard inoculum was 0.2 cc. of a suspension of viable organisms in a concentration of 30 organisms per oil-immersion field.

Six chickens were inoculated, 3 by intravenous injection and 3 by intrathoracic injection. They were sacrificed after 10 weeks. Lesions were not found in any of these animals.

Six rabbits were inoculated, 2 by intravenous, 2 by intraperitoneal, and 2 by subcutaneous injection. One rabbit, intravenously inoculated, died 3 weeks later of septicemia, staphylococci being recovered from the blood stream. The site of inoculation showed no reaction. The remaining 5 rabbits were skin-tested and sacrificed after 10 weeks. The average weight gain was 655 gm. Lesions were not found in any of these animals.

Twelve goldfish were used. In 6 the inoculum was injected intraperitoneally, and in 4 it was injected into the dorsal lymph sac. Lesions failed to form in all cases.

Six frogs, *Rana cetesbiana,* were used. In 3 the inoculum was injected intraperitoneally, and in 3 it was injected into the dorsal lymph sac. Lesions failed to form in all cases.

Ten rats were used: 5 were injected subcutaneously and 5 intraperitoneally. Among the group injected subcutaneously there was one which died 1 week later. Its body was not recovered. The remaining 4 were sacrificed 10 weeks later after a negative skin test. These animals showed an average weight gain of 120.9 gm. In one there was proliferation of lymphoid tissue in the spleen, in which acid-fast organisms could be stained. Two other rats showed focal accumulations of epithelioid cells in the lymph nodes. In these foci acid-fast organisms were stained, some of them showing "Y" forms (Fig. 19). The last rat had no lesions. Among the group inoculated by intraperitoneal injection, one rat died of pneumonia in 8 days. Proliferation of macrophages with giant cell formation occurred at the site of inoculation. Acid-fast organisms were stained, some of which were "Y" forms. The remain-

TABLE I

Species-Susceptibility to the Organism

Animal	Route of infection	Weight		Sensitivity	Death	Significant lesions	Organisms stained in tissues
		Initial	Death				
		gm.	*gm.*				
Mouse 1	Subcutaneous	15.6	21.3	Negative	Sacr. 72 days	None	No
Mouse 2	Subcutaneous	14.8	21.0	Not done	Died 33 days	Pneumonia, granuloma of lymph nodes	Yes
Mouse 3	Subcutaneous	14.4	16.7	Negative	Died 40 days	Pneumonia, granuloma of liver	Yes
Mouse 4	Subcutaneous	14.3	16.5	Negative	Sacr. 72 days	None	No
Mouse 5	Subcutaneous	14.8	17.9	Negative	Died 45 days	Pneumonia, focal necrosis of liver	No
Mouse 6	Intraperitoneal	11.4	9.7	Not done	Died 26 days	Granuloma of intestines, liver, lymph node	Yes
Mouse 7	Intraperitoneal	8.4	7.5	Not done	Died 6 days	Focal necrosis of liver, hemorrhage in abdomen	No
Mouse 8	Intraperitoneal	9.4	9.4	Not done	Died 1 day	Peritoneal hemorrhage	No
Mouse 9	Intraperitoneal	13.2	13.2	Not done	Died 2 days	Granuloma, mesenteric lymph nodes	Yes
Mouse 10	Intraperitoneal	12.3	11.6	Negative	Died 34 days	None, focal necrosis of liver	No
Rat 1	Subcutaneous	27.8	26.6	Not done	Died 7 days(?)	No remains	No
Rat 2	Subcutaneous	48.0	191.6	Negative	Sacr. 69 days	Granuloma in spleen	Yes
Rat 3	Subcutaneous	35.0	134.2	Doubtful	Sacr. 69 days	Granuloma of lymph node	Yes
Rat 4	Subcutaneous	67.2	192.2	Doubtful	Sacr. 69 days	None	No
Rat 5	Subcutaneous	44.3	160.2	Negative	Sacr. 69 days	Granuloma of lymph node	Yes
Rat 6	Intraperitoneal	67.2	192.2	Doubtful	Sacr. 69 days	Granuloma of lymph node	Yes
Rat 7	Intraperitoneal	29.8	182.7	Negative	Sacr. 69 days	None	No
Rat 8	Intraperitoneal	46.5	74.8	Not done	Died 8 days	Granuloma at site of inoculation	Yes
Rat 9	Intraperitoneal	44.9	161.5	Positive	Sacr. 69 days	Granuloma of lymph nodes	Yes
Rat 10	Intraperitoneal	40.3	150	Doubtful	Sacr. 69 days	None	No
Guinea-pig 1*	Subcutaneous	575	690	Positive	Sacr. 70 days	Granuloma at site of inoculation	Yes
Guinea-pig 2*	Subcutaneous	380	525	Positive	Sacr. 70 days	Granuloma, lymph nodes and liver	Yes
Guinea-pig 3*	Subcutaneous	350	450	Positive	Sacr. 70 days	Granuloma, lymph nodes, epididymis, and liver	Yes
Guinea-pig 4*	Subcutaneous	420	575	Positive	Sacr. 70 days	Granuloma, liver and gastrointestinal tract	Yes

Guinea-pig 5*	Subcutaneous	535	650	Positive	Sacr. 70 days	Granuloma, epididymis and lymph nodes	Yes
Guinea-pig 6*	Subcutaneous	515	600	Positive	Sacr. 70 days	Granuloma, lymph nodes, liver, and brain	Yes
Rabbit 1908	Intravenous	2175	1575	Not done	Died 21 days	Pneumonia, focal necrosis of liver	No
Rabbit 1911	Intravenous	2325	3150	Negative	Sacr. 68 days	None	No
Rabbit 1913	Intraperitoneal	2025	2900	Negative	Sacr. 68 days	None	No
Rabbit 1919	Intraperitoneal	2275	3100	Negative	Sacr. 68 days	None	No
Rabbit 1922	Subcutaneous	1925	2250	Negative	Sacr. 68 days	None	No
Rabbit 1923	Subcutaneous	2375	2900	Negative	Sacr. 68 days	None	No
Chicken 1	Intravenous				Sacr. 69 days	None	No
Chicken 2	Intravenous				Sacr. 69 days	None	No
Chicken 3	Intravenous				Sacr. 69 days	None	No
Chicken 4	Intrapleural				Sacr. 69 days	None	No
Chicken 5	Intrapleural				Sacr. 69 days	None	No
Chicken 6	Intrapleural				Sacr. 69 days	None	No
Frog 1	Dorsal lymph sac				Died 6 days	None	No
Frog 2	Dorsal lymph sac				Sacr. 60 days	None	No
Frog 3	Dorsal lymph sac				Sacr. 60 days	None	No
Frog 4	Intraperitoneal				Died 20 days	None	No
Frog 5	Intraperitoneal				Sacr. 60 days	None	No
Frog 6	Intraperitoneal				Died 28 days	None	No
Goldfish 1	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 2	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 3	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 4	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 5	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 6	Intraperitoneal				Sacr. 92 days	None	No
Goldfish 7	Tail				Sacr. 92 days	None	No
Goldfish 8	Tail				Sacr. 92 days	None	No
Goldfish 9	Tail				Sacr. 92 days	None	No
Goldfish 10	Tail				Sacr. 92 days	None	No
Goldfish 11	Organisms in water				Sacr. 92 days	None	No
Goldfish 12	Organisms in water				Sacr. 92 days	None	No

* A moderate swelling appeared at site of inoculation, which receded during the third week.

ing 4 rats were sacrificed after 10 weeks. The average weight gain was 131.9 gm. Two of these animals showed focal accumulations of epithelioid cells in the lymph nodes, from which acid-fast organisms were re-isolated. The other 2 showed no lesions.

Ten mice were used; 5 were injected subcutaneously, and 5 intraperitoneally. In the first group one died at 5 weeks. This animal showed small collections of epithelioid cells in the lymph nodes. Death was due to pneumonia. A second mouse died of pneumonia, also. There were focal collections of macrophages in the lymph nodes with focal necrosis in the liver, but acid-fast organisms could not be demonstrated. A third mouse died after 7 weeks. No lesions were found which could be ascribed to infection by acid-fast organisms. Two mice lived 10 weeks, whereupon they were sacrificed but no lesions were found. The average weight gain was 3.95 gm. Among the group intraperitoneally injected, 2 died by the second day of hemorrhage. In one, acid-fast organisms formed a focal aggregate in a mesenteric lymph node. No other lesions were found. A third mouse died at 6 days of peritoneal hemorrhage. A fourth died at 4 weeks. In this mouse there was macrophagic proliferation in the mesenteric nodes and those surrounding the intestine, with small tubercle-like lesions containing acid-fast organisms in the liver. A fifth mouse died at 5 weeks. No lesions referable to acid-fast organisms were found, but there was focal necrosis in the liver.

Six guinea-pigs were injected subcutaneously. In each a large swelling characterized by an epithelioid reaction of large macrophages and by central necrosis appeared at the site of injection. Acid-fast organisms invariably were isolated in cultures and were stained in histologic sections. Lesions in the guinea-pig differed from those in the patient in that, while the acid-fast organisms usually were arranged in clumps, massive intracellular parasitism was not found. The inguinal lymph nodes in 3 and the tracheobronchial lymph nodes in 4 of the 6 guinea-pigs showed a granulomatous reaction in the form of marked hyperplasia of the macrophages. In these nodes acid-fast organisms were stained. In 4 animals a reaction of the same kind was found in the liver (Fig. 20). These granulomas consisted of an aggregation of macrophages with a central area of necrosis. Inflammatory cells of other types were rare. Three animals showed diffusely scattered areas of focal necrosis of the liver. The lymphoid elements of the gastrointestinal tract showed uniformly a proliferative reaction in which acid-fast organisms were stained in only 2 instances. The lungs also uniformly showed proliferation of the lymphoid elements surrounding the vessels and the bronchi (Fig. 21), but in only one lung were acid-

fast organisms stained. In one guinea-pig there was a characteristic lesion in the wall of the gallbladder, and also lesions appeared in the epididymis (Fig. 22). In one guinea-pig a small group of epithelioid cells was found in the meninges of the basal portion of the brain. In this tubercle-like lesion acid-fast organisms were stained. There was one instance of epithelioid reaction in the spleen in which acid-fast organisms were found; however, in all animals there was a diffuse proliferation of lymphoid elements. Lesions did not appear in the heart, kidney, or adrenal of any inoculated guinea-pig.

Route of Infection

For the purpose of investigating the route of infection, guinea-pigs were selected. Groups of 4 animals were inoculated in each of the following ways: By intravenous, intraperitoneal, and subcutaneous injection; by ingestion; and by instillation into the conjunctival sac (Table II).

In the group inoculated intravenously, the lungs showed proliferation of lymphoid elements in 3 animals, with the formation in one of a tubercle-like lesion carrying acid-fast organisms. One guinea-pig showed no lesion in the lung. Lymph nodes in 2 showed hyperplasia of the reticulo-endothelial elements, but carried no acid-fast organisms. In the livers there were 2 instances of marked reaction in the form of proliferation of macrophages with a central area of necrosis, one of phagocytosis of acid-fast organisms by the Kupffer cells, and one in which there was no lesion. The spleens of these animals showed uniform proliferation of the lymphoid elements, and in one instance acid-fast organisms were seen intracellularly. One of these guinea-pigs showed a granulomatous lesion in the epididymis typical of the kind produced by this organism.

In the group receiving intraperitoneal injections there was uniform hyperplasia of the lymphoid elements in the lungs, lymph nodes, and spleens. In the liver of one of these animals there were groups of epithelioid cells containing scattered acid-fast organisms. In 2 animals the same lesion appeared in the lymph nodes, but the fourth animal showed no lesions, and no acid-fast organisms were stained.

In the group inoculated by subcutaneous injection there was produced uniformly an area of necrosis surrounded by large mononuclear macrophages at the site of inoculation. With the exception of regional inguinal nodes, the lymph nodes were hyperplastic in only one instance. In the livers of 2 animals were found lesions, consisting of large mononuclear phagocytes, with a central area of necrosis. Acid-fast organisms

TABLE II

Route of Infection

Guinea-pig no.	Route of infection	Weight		Sensitivity	Sacrificed after	Significant lesions	Organisms stained
		Initial	Death				
		gm.	*gm.*				
1876	Intravenous	700	625	++	84 days	Granuloma of epididymis	Yes
1890	Intravenous	475	450	++	84 days	Granuloma of lungs and liver	Yes
1882	Intravenous	725	700	++	84 days	Phagocytosis by Kupffer cells and macrophages of spleen	Yes
1895	Intravenous	500	450	++	84 days	Granuloma of liver	Yes
1879	Intraperitoneal	400	600	++	81 days	None	No
1892	Intraperitoneal	400	550	++	81 days	Granuloma of lymph node	Yes
1900	Intraperitoneal	400	675	++	81 days	Phagocytosis of acid-fast organisms in lymph nodes	Yes
1913	Intraperitoneal	325	700	++	81 days	Tuberculoid reaction in liver	Yes
1918*	Subcutaneous	650	675	++	84 days	Granuloma of liver	Yes
1924*	Subcutaneous	675	700	++	84 days	None	Yes
1901*	Subcutaneous	725	925	++	84 days	Healing lesion at site of inoculation	No
1902*	Subcutaneous	300	400	++	84 days	Granuloma of liver	Yes
1906	Ingestion	625	725	o	82 days	Granuloma of liver, lungs, intestines	Yes
1921	Ingestion	350	525	++	82 days	Acid-fast organisms stained in liver	Yes
1905	Ingestion	400	550	++	82 days	Granuloma of lungs and lymph nodes	Yes
1903	Ingestion	425	500	++	82 days	Acid-fast organisms stained in lungs, liver, and lymph nodes	Yes
1907	Instillation, eye	500	650	?	85 days	None	No
1909	Instillation, eye	775	725	++	85 days	Granuloma of lungs, acid-fast organisms stained in spleen	Yes
1915	Instillation, eye	375	375	?	85 days	None	No
1920	Instillation, eye	350	460	++	85 days	Granuloma of lymph node and spleen	Yes

* Showed granulomatous inflammation at site of inoculation. The lesion was healed at autopsy.

were stained in the necrotic areas. The spleen was uniformly hyperplastic, but no acid-fast organisms were found. None of these animals showed lesions in the epididymis.

In all 4 of the animals which had ingested the inoculum the quantity of lymphoid tissue in the lungs was striking. Three showed lymph nodes with extensive proliferation of macrophages, and in 2 areas of proliferation many acid-fast organisms were stained. These organisms did not occupy an intracellular position. The livers of 2 guinea-pigs showed acid-fast organisms within the Kupffer cells, but no granulomatous lesions. Two animals showed extensive proliferation of the lymphoid tissue of the spleen, and in one spleen acid-fast organisms were stained. The intestines of 2 guinea-pigs showed marked proliferation of the lymphoid follicles, and, in one, acid-fast organisms were demonstrated in the follicles. The kidneys of 2 animals showed nodules of epithelioid cells in which acid-fast organisms were found.

Only 2 of the 4 animals which had received instillation of inoculum into the conjunctival sac showed infection. In one there was conspicuous lymphoid proliferation in the lymph nodes and spleen, the latter containing acid-fast organisms. The other, in addition, showed a granulomatous reaction in the lung. This lesion consisted of a marked proliferation of the large macrophages with acid-fast organisms scattered throughout.

All of the animals used in this experiment were inoculated intradermally with a suspension of organisms killed by autoclaving. This inoculation produced a reaction about 24 hours after the injection. The reaction consisted of swelling and induration with a broad area of erythema. In the 2 guinea-pigs which resisted infection by ingestion of the inoculum there was no reaction. Histologically, the sites of reaction consisted of a central zone of necrosis and liquefaction, with polymorphonuclear leukocytes. In the focus of polymorphonuclear leukocytes numerous fragmented, acid-fast granules were demonstrated. Surrounding this zone of polymorphonuclear response there was extensive proliferation of macrophages, some of which contained large numbers of acid-fast granules. There was, in addition, an infiltration by lymphocytes and eosinophils with fibroblastic proliferation.

Development of the Lesion and Course of the Infection

For the purpose of studying the development of the lesion and course of the infection, 18 mice and 24 guinea-pigs were used. These animals were inoculated subcutaneously with 0.2 cc. of a suspension containing approximately 30 living organisms per oil-immersion field. They were sacrificed, 2 at a time, at weekly intervals (Table III).

TABLE III

Course of the Infection *

Animal	Sensitivity	Death	Significant lesions	Organisms stained in tissues
Guinea-pig A1	Negative	Sacr. 9 days	Healing scar at site of inoculation	No
Guinea-pig A2	Negative	Sacr. 9 days	Healing scar at site of inoculation	No
Guinea-pig A3	Not done	Died 11 days	Pneumonia; healing at site of inoculation	No
Guinea-pig A4	Not done	Died 11 days	Healing at site of inoculation	No
Guinea-pig A5	Not done	Died 11 days	Healing at site of inoculation	No
Guinea-pig A6	Not done	Died 12 days	Pneumonia; healing at site of inoculation	No
Guinea-pig A7	Negative	Sacr. 16 days	Nothing	No
Guinea-pig A8	Negative	Sacr. 16 days	Nothing	No
Guinea-pig A9	Not done	Died 18 days	Pneumonia; healing at site of inoculation	Yes
Guinea-pig A10	Not done	Died 18 days	Pneumonia; healing at site of inoculation	Yes
Guinea-pig A11	Not done	Died 18 days	Pneumonia; healing at site of inoculation	No
Guinea-pig A12	Not done	Died 18 days	Nothing	No
Guinea-pig A13	Not done	Died 21 days	Nothing	No
Guinea-pig A14	Not done	Died 23 days	Pneumonia	No
Guinea-pig A15	Negative	Sacr. 23 days	Nothing	No
Guinea-pig A16	Negative	Sacr. 23 days	Nothing	No
Guinea-pig A17	Negative	Sacr. 30 days	Granuloma of lymph nodes	Yes
Guinea-pig A18	Negative	Sacr. 30 days	Focal proliferations in lymph nodes	No
Guinea-pig A19	Not done	Died 32 days	Pneumonia	No

Guinea-pig A20	Doubtful	Sacr. 37 days	Focal proliferation in lymph nodes	Yes
Guinea-pig A21	++	Sacr. 37 days	Granuloma of lymph nodes and liver	Yes
Guinea-pig A22	++	Sacr. 42 days	Granuloma of lymph nodes and necrosis of liver	Yes
Guinea-pig A23	++	Sacr. 42 days	Peculiar macrophagic response in lungs	No
Guinea-pig A24	++	Sacr. 42 days	Nothing	No
Mouse A1	Not done	Died 1 day	Necrosis of liver	No
Mouse A2	Not done	Died 7 days	Granuloma of skin at site of inoculation	Yes
Mouse A3	Negative	Sacr. 9 days	Granuloma of skin at site of inoculation	Yes
Mouse A4	Negative	Sacr. 9 days	Granuloma of skin at site of inoculation	Yes
Mouse A5	Not done	Died 11 days	Healing skin lesion at site of inoculation	No
Mouse A6	Negative	Sacr. 16 days	Healing skin lesion at site of inoculation and lymph node	Yes
Mouse A7	++	Sacr. 16 days	Healing skin lesion at site of inoculation	No
Mouse A8	+++	Sacr. 23 days	Healing skin lesion at site of inoculation and lymph nodes	Yes
Mouse A9	+	Sacr. 23 days	Healing skin lesion at site of inoculation	Yes
Mouse A10	+	Sacr. 30 days	Nothing	No
Mouse A11	+	Sacr. 30 days	Nothing	No
Mouse A12	Not done	Died 33 days	Nothing	No
Mouse A13	++	Sacr. 37 days	Nothing	No
Mouse A14	++	Sacr. 37 days	Nothing	No
Mouse A15	o	Sacr. 44 days	Granuloma of lymph node	Yes
Mouse A16	+	Sacr. 44 days	Scars in spleen and lymph node	No
Mouse A17	o	Sacr. 51 days	Nothing	No
Mouse A18	++	Sacr. 51 days	Nothing	No

* All animals developed a swelling at the site of inoculation. This swelling gradually subsided after about 2 to 3 weeks.

The mice uniformly showed a slight swelling and occasionally ulceration at the site of inoculation (Fig. 23). Biopsy of one of these lesions showed the characteristic reaction, described in the preceding section. In the areas of necrosis were many acid-fast organisms. Mice which were sacrificed on the 11th day showed disappearance of the central area of necrosis and replacement by a granulomatous reaction of a different form, consisting of large mononuclear phagocytes with Langhans' giant cells. Those sacrificed on the 23rd day showed a granulomatous reaction in the liver and spleen. In those sacrificed on the 30th day, the site of inoculation was completely healed, and in its place only scar tissue was found. Internally these mice showed only extensive hyperplasia of the lymphoid elements of the liver and lymph nodes. Acid-fast organisms were not found. In none of the mice sacrificed subsequently were active lesions found, with the exception of one sacrificed on the 44th day. In this mouse there was an extensive macrophagic proliferation of the regional lymph nodes. Acid-fast organisms were found in an intracellular position in these nodes. In one mouse a large area of scar tissue was found in the spleen. This was thought to be a healed lesion. Three of these mice showed healed, tubercle-like lesions in the liver.

The guinea-pigs, like the mice, exhibited a granulomatous reaction at the site of inoculation. Those sacrificed between the 9th and the 18th day showed healing at the site of inoculation. Guinea-pigs sacrificed on the 30th and 37th days showed internal granulomatous reactions; 2 had focal epithelioid proliferation in the lymph nodes. One showed a lesion of this type in the liver, and proliferation of the reticulo-endothelial elements of the lung, which contained acid-fast organisms. In all 4 there was hyperplasia of the lymphoid elements of the spleen. One animal, sacrificed on the 42nd day, showed a granulomatous reaction in a lymph node in which acid-fast organisms were stained. Acid-fast organisms also were stained in the lung, and there were extensive areas of necrosis in the liver. All guinea-pigs sacrificed subsequent to the 37th day were without active lesions.

The course of infection in the experimental animal was different from that in the patient. At the site of inoculation there developed an area of necrosis surrounded by large mononuclear macrophages. This lesion healed within about 3 weeks. Throughout the phase of healing the lesion was characteristically granulomatous, with proliferation of macrophages and formation of giant cells. The organism occasionally would be found in a "Y" form in such lesions. The infec-

tion usually remained localized. However, in many animals small isolated lesions were produced in lungs, liver, spleen, and intestine. In the course of 4 to 5 weeks all lesions healed, and sensitivity of the tuberculin type was produced. The infection did not kill the animal.

The guinea-pigs used in this experiment were tested by intradermal inoculation of organisms killed by heat. No sensitivity developed until the 30th day of the infection. These animals had a slight reaction, and all sacrificed subsequently showed a reaction of the tuberculin type, which became distinct by the 42nd day.

Summary of the Studies on the Pathogenicity of Nocardia intracellularis, n. sp.

Nocardia intracellularis, n. sp., was found to be pathogenic * for guinea-pigs, rats, and mice, but it produced no lesion in rabbits, chickens, frogs, or goldfish. Infection of guinea-pigs by this organism was successful when it was injected subcutaneously, intravenously, and intraperitoneally, and when fed by mouth; but when it was instilled into the conjunctival sac infection was successful in only 50 per cent of animals. The animals for which *N. intracellularis, n. sp.*, is pathogenic uniformly showed a local reaction at the site of inoculation consisting of proliferation of macrophages with necrosis in the center of the lesion but with little intracellular parasitism. Ten per cent of these animals had a systemic distribution of the lesions, chiefly in the liver, lungs, and lymph nodes. These lesions healed after about 6 weeks, at which time the animals exhibited a tuberculin-like skin sensitivity to a heat-killed suspension of the organism.

General Summary and Discussion

In summary, the condition described is a new granulomatous disease entity caused by an unusual acid-fast organism. The source of the infection is not known. Inasmuch as the most numerous and the largest lesions were found in the retroperitoneal and mesenteric lymph nodes and in the lymphoid tissue of the intestines, the gastro-intestinal tract is suggested as the portal of entry. The characteristic reaction to the organism consisted of phagocytosis of the pathogen by large mononuclear phagocytes with proliferation of these cells and the production of multinucleated giant cells. The only other basic pathologic process represented was necrosis of a coagulative type. In the absence of poly-

* For the purpose of this report, pathogenic is defined as "capable of producing a disturbance in function or structure of any organ or part of the body" and does not imply that the disease must be lethal.

morphonuclear leukocytes and even lymphocytic infiltration, this lesion may be regarded as a pure form of granuloma.[31] This granuloma exhibited no consistent morphologic structure such as tubercle formation, but rather presented itself simply as a diffuse proliferation of macrophages.

In spite of its ability to resist the digestive activity of macrophages and to multiply to an extraordinary degree within these cells, the organism appeared to elaborate little that was toxic to its host. The damage done by it seemed to arise solely out of its ability to grow and multiply within reticulo-endothelial cells of the host, which relationship gave rise to an extraordinary and almost unlimited proliferation of these cells. The initial reaction to this organism was phagocytosis, followed by proliferation of the phagocytes. The end-result of this ultimate form of parasitism is death of the host cells.

The major systemic effects seemed to depend upon the specific location of the larger and more numerous lesions. Their position in the lymphatic system of the gastro-intestinal tract produced obstruction of the lymphatics and consequently failure of absorption of fat. This failure was reflected in the engorged state of the lymphatics and had produced, in part, the systemic effect of inanition. In addition to the nutritional disturbance, there may be other factors in the production of inanition. The extreme degree of proliferation of the macrophages and the clinical course suggested a neoplasm. Indeed, had the presence of organisms not been demonstrated, one would have been justified, from the history and gross examination of the lesion, in considering this disease a peculiar form of lymphosarcoma. In view of this superficial similarity to a neoplasm, the other unknown factors in the production of death might be similar to those with cancer.

Placement of this organism within an established classification has been exceedingly difficult. The degree of acid-fastness and its ability to withstand sodium hydroxide digestion have suggested that it is related to the mycobacteria. However, that it branches consistently and early, that it grows on Sabouraud's medium at room temperature, that it utilizes paraffin as a sole source of carbon, and that it grows rapidly and well on a multiplicity of media seem to identify it more closely with the actinomycetes. Our difficulty in choosing between these two groups is in harmony with the generally prevailing uncertainty of the position of acid-fast actinomycetes. Many have considered them to be mycobacteria, and many have considered mycobacteria to be fungi.[32-35] Into this controversy we prefer not to enter, but the organism seems

most closely related to the genus designated Nocardia, which for the time being is assigned to the family Actinomycetaceae. Since we have been unable to find a reported species with which it is identical, we have concluded that this organism represents an undescribed species of Nocardia. Because its presence within the cells whose reactivity it provokes in its human host is so constant, so typical, and so impressive, we have utilized this characteristic in assigning the name *Nocardia intracellularis, n. sp.,* to it.

Lesions have been produced by this organism in guinea-pigs, rats, and mice, but not in rabbits, chickens, frogs, or goldfish. The inoculation lesion consists of a central zone of necrosis in which groups of organisms may be found. This zone is surrounded by macrophages, in which there are very few organisms. On the outer edge of this macrophagic zone is fibroblastic proliferation. In only about 10 per cent of infections is there systemic spread. When found, the systemic lesions are histologically similar to the local lesion. There have been no fatal infections in these animals. The chief difference between the reaction in the experimental animal and in man is the relative paucity of intracellular organisms and the relative abundance of necrosis in the experimental animal.

The organism under consideration has been contrasted with 37 other acid-fast actinomycetes with respect to the lesion which they produce and the animals for which they are pathogenic (Table IV). Thirty of these organisms were isolated from human sources. Nineteen of these were obtained from abscesses, of which 13 occurred in the lungs. Draining sinuses, including those of madura foot, were the source of three of these organisms. One was obtained from a case of postoperative peritonitis. Three were found in pulmonary lesions which were called bronchopneumonia, and one was isolated from "cirrhotic nodules of the lungs." There was one from a case "resembling plague," one from a case of "fibrosis of the spleen," and one from a patient who did not die, but whose x-ray findings suggested tuberculosis.

From this summary of cases it is seen that acid-fast actinomycetes have been isolated from a wide variety of human lesions. The only case said to be granulomatous was that of Gammel,[25] but the lesions which he described were not similar to those of the patient here presented. In fact, we have been unable to find in the literature a description of any case which is essentially similar.

All of the 37 organisms referred to above show a striking variability in pathogenicity for animals. The variation ranged from lethal patho-

TABLE IV

Source and Animal Pathogenicity of Nocardia intracellularis, n. sp., Contrasted with 37 Reported Acid-Fast Actinomycetes

Author	Name of organism	Source	Pathogenic for	Nonpathogenic for
Eppinger[15]	*A. asteroides*	Abscess of lungs and brain	Guinea-pig, rabbit	Mouse
Aoyama and Miyamoto[36]	*A. asteroides*	Abscess of lungs	Guinea-pig	Fowl, rabbit, mouse
Buchholz[37]	*A. asteroides*	Abscess of lungs	Not stated	
Foulerton[34]	*A. asteroides*	Abscess of lungs		Guinea-pig
Horst[38]	*A. asteroides*	Abscess of lungs	Guinea-pig, rabbit	Mouse
Loehlein[39]	*A. asteroides*	Abscess of heart, lungs, brain	Guinea-pig, rabbit	Dog
MacCallum[40]	*A. asteroides*	Postoperative peritonitis	Mouse, dog, guinea-pig, rabbit	
Schabad[41]	*A. asteroides*	Abscess of lungs and chest wall	Guinea-pig, rabbit	Pigeon, mouse
Stokes[4]	*A. asteroides*	Abscess of lungs	Guinea-pig, rabbit	
Birt and Leishman[17]	*A. asteroides**	"Cirrhotic nodules" of lung	Guinea-pig	
Musgrave and Clegg [42]	*A. asteroides*†	Madura foot	Dog, monkey, guinea-pig	Rabbit
Sabrazès and Rivière[43]	*A. asteroides*(?)	Abscess of brain	Guinea-pig(?), rabbit(?)	Rat
Sabrazès and Rivière[43]	*A. asteroides*(?)	Pleuropneumonia		
Berestneff[19]	*A. rivierei* (Brumpt)	Abscess of brain	Guinea-pig(?), mouse(?), dog(?), rabbit(?)	
Vuillemin[22]	*A. jollyi*	Case resembling plague	Not stated	
Butterfield[44]	*A. anaerobies*	Abscess of lungs	Not stated	
De Korté[45]	*A. cylindraceus*	Draining sinus of ear	Not stated	
Flexner[46]	*A. pseudotuberculosis*	Abscess of lungs	Guinea-pig(?)	
Gammel[25]	*A. phenotolerans*	Acne of face	Guinea-pig	
Gibson[47]	*A. gibsoni*	Fibrosis of spleen	Not stated	
Greco[48]	*A. sommeri*	Mycetoma pedis	Rabbit, guinea-pig	
Henrici and Gardner[16]	*A. gypsoides*	Bronchopneumonia	Guinea-pig	
Ferré and Faguet[49]	*A. sabrazes*(?)	Cerebral abscess	None	Rabbit, guinea-pig
Bernstein[50]	Unnamed	Abscess of lungs	Guinea-pig, rabbit	
Davis[21]	Unnamed	Sputum in case with radiograph similar to tuberculosis	Rabbit, mouse	
Davis and Garcia[23]	Unnamed	Subcutaneous abscess	Rabbit, guinea-pig, rat, mouse	
Goldsworthy[26]	Unnamed	Lung abscess		
Ljubimoff[51]	Unnamed	Bronchopneumonia	Not stated	
Scheele and Petruschky[52]	Unnamed	Abscesses of lung and skin	Guinea-pig(?), rabbit(?)	
Ribytkof and Maloletkof[53]	Unnamed	Abscess of spinal cord		
De Mello and St. Antonio Fernandes[54]	*A. chalmersi*	Saliva of horse	Not stated	

Lombardo-Pellegrino[55]	*A. viridis*	Soil	Rabbit, guinea-pig, cat	Rabbit, dog, cat, horse
Nocard[13]	*A. farcinicus*	Chronic suppurative disease of cattle	Guinea-pig	
Rabe[56]	*A. canis*	Abscess and draining sinus in neck and forepaw of dog	Rabbit, guinea-pig	
Redaelli[57]	*A. sanfelicei*	Rat	Guinea-pig, rabbit, rat, dog	Fowl
Silberschmidt[58]	*A. caprae*	Lung of goat	Rabbit, guinea-pig	Mouse
Trolldenier[59]	Unnamed	Caseopurulent abscess of dog		
Cuttino and McCabe	*N. intracellularis, n. sp.*	Granulomatous lesion in lymph nodes and spleen	Guinea-pig, rat, mouse	Rabbit, fowl, frog, goldfish

* Named *A. leishmani* by the authors; later identified as *A. asteroides* by Foulerton.[34]
† Named *A. freeri* by the authors; later identified as *A. asteroides* by the authors.

genicity for all animals tested to nonpathogenicity for any laboratory animal. Here again, none of them reacted exactly like the organism under consideration.

Conclusions

A new disease entity in man was characterized clinically by an abdominal mass, malnutrition, and an afebrile course. The morphologic, cultural, and biochemical characteristics of the causative organism, when contrasted with those of other organisms, present significant differences in every instance, justifying the designation of this organism as a new species to which the name *Nocardia intracellularis, n. sp.,* has been given. This organism is assigned to the genus Nocardia, family Actinomycetaceae.

In man the disease produced by this organism is a pure form of granulomatous inflammation, characterized by phagocytosis of the pathogen by reticulo-endothelial cells and proliferation of these cells. In the spleen and lymph nodes the proliferation of macrophages is of such proportion as to displace completely the normal structure. There is equilibrium between the organism and its host cell to the extent that massive intracellular parasitism constitutes perhaps the most distinctive feature of the disease. Comparatively little necrosis occurs, and there is no response on the part of polymorphonuclear leukocytes and other inflammatory elements customarily found in the common infectious granulomas.

Nocardia intracellularis, n. sp., produces a nonlethal disease in guinea-pigs, rats, and mice. It produces no lesions in chickens, rabbits, frogs, and goldfish.

In the experimental animal, *Nocardia intracellularis, n. sp.,* provokes a response which

differs sharply from the reaction in man. This difference is to be found in comparatively extensive necrosis and relative paucity of intracellular organisms. The lesions heal rapidly in the experimental animal and are accompanied by the production of sensitivity of the tuberculin type.

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[*Illustrations follow*]

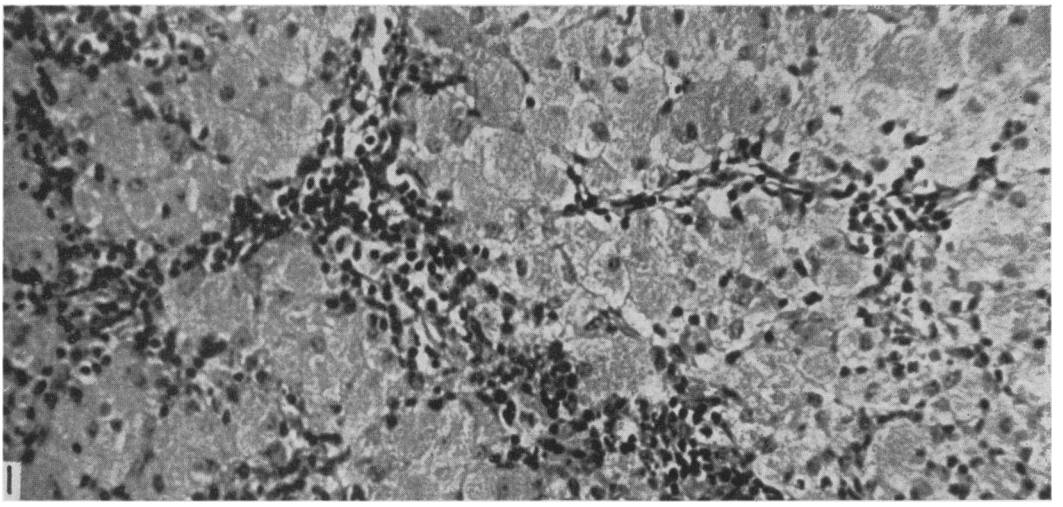
DESCRIPTION OF PLATES

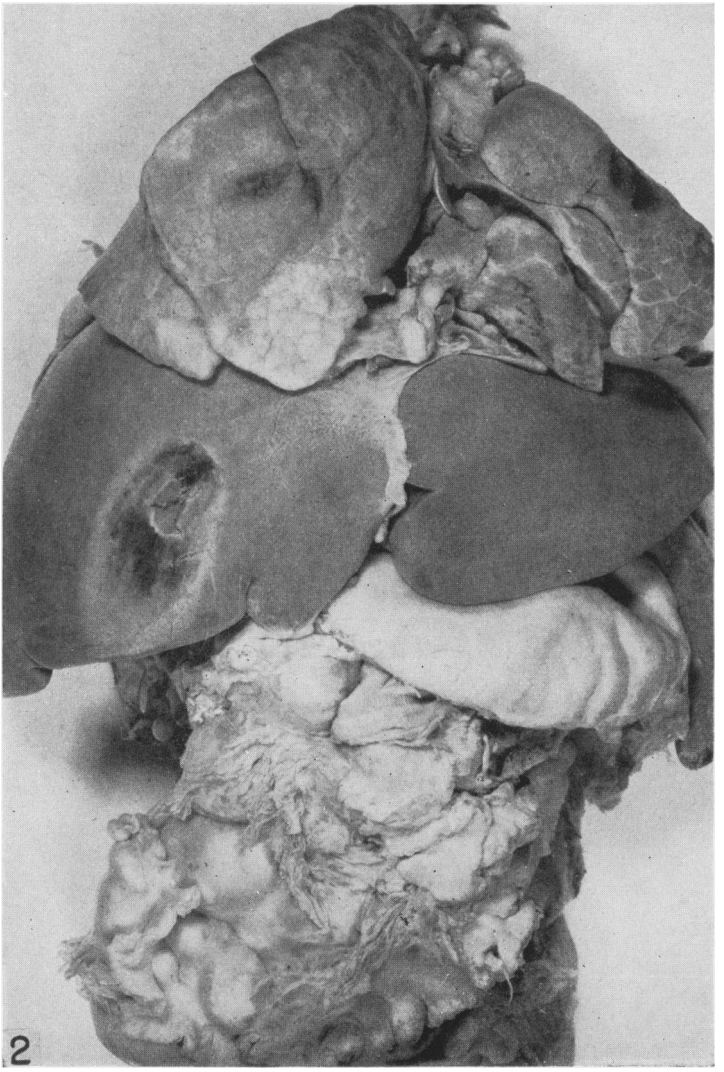
PLATE 1

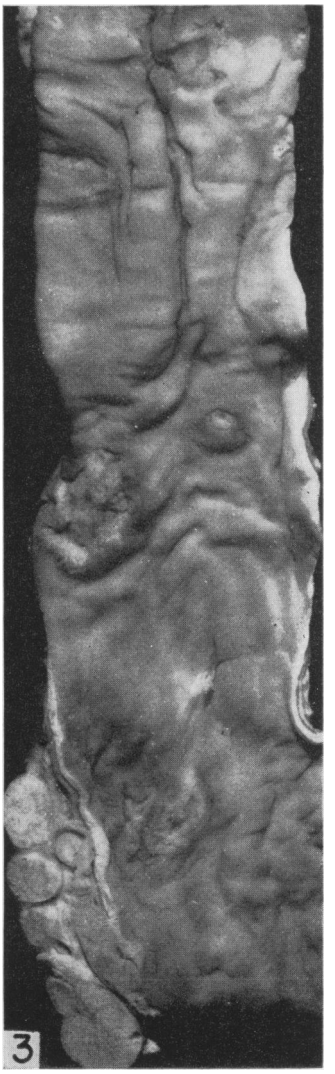
FIG. 1. From the original lymph node, removed for biopsy, showing widespread replacement of lymphoid tissue by large foamy macrophages. Hematoxylin and eosin stain. × 290.

FIG. 2. Thoracic and abdominal viscera, showing the mass of enlarged and matted lymph nodes of the mesenteric and periaortic groups. There is also enlargement of the left subclavian nodes. The spleen, partially visible at the right, likewise is enlarged. The discoloration of the liver and lungs is an artifact.

FIG. 3. Colon showing large, irregular ulcers with necrotic bases. The intervening mucosa has lost its normal corrugation. The marginal lymph nodes show enlargement due to proliferation of macrophages.







Cuttino and McCabe Granulomatous Nocardiosis

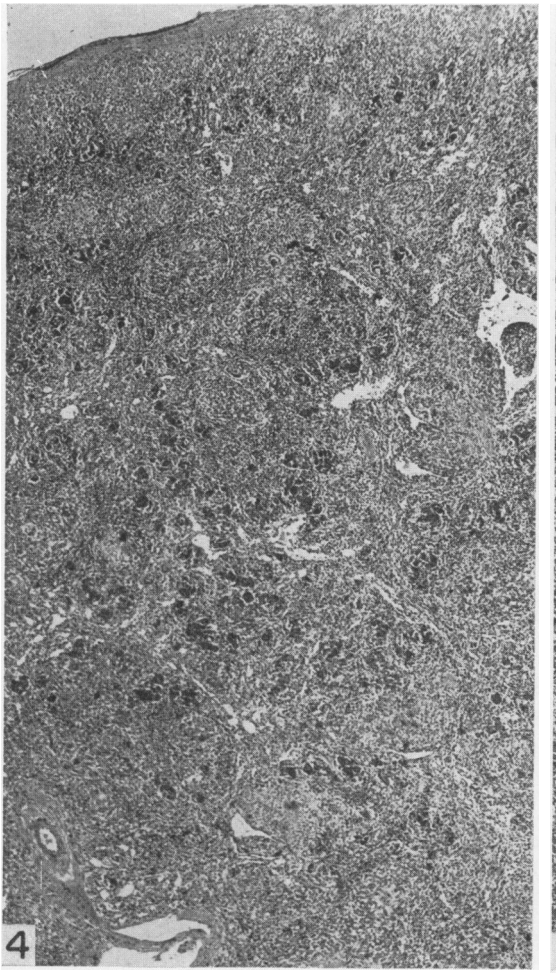
PLATE 2

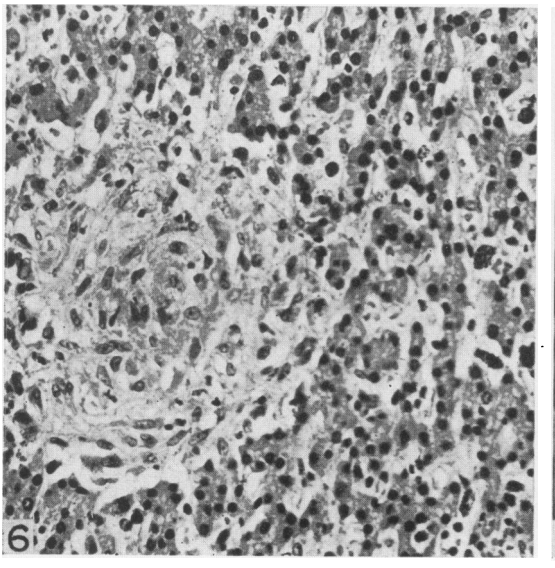
FIG. 4. Spleen with complete replacement of its structure by macrophages. The large, deeply stained bodies are multinucleated giant cells. Hematoxylin and eosin stain. × 40.

FIG. 5. Mesenteric lymph node with the same changes as were found in the spleen (Fig. 4). In both lymph node and spleen there is absence of necrosis, but the formation of giant cells is more impressive in the lymph node. Hematoxylin and eosin stain. × 40.

FIG. 6. A tuberculoid nodule in the liver. There is also enlargement of Kupffer cells. Hematoxylin and eosin stain. × 300.

FIG. 7. A higher power view of the same nodule seen in Figure 6. It is stained to show the great number of intracellular, acid-fast organisms, both in cells of the nodule and in isolated Kupffer cells. Ziehl-Neelsen's stain. × 725.





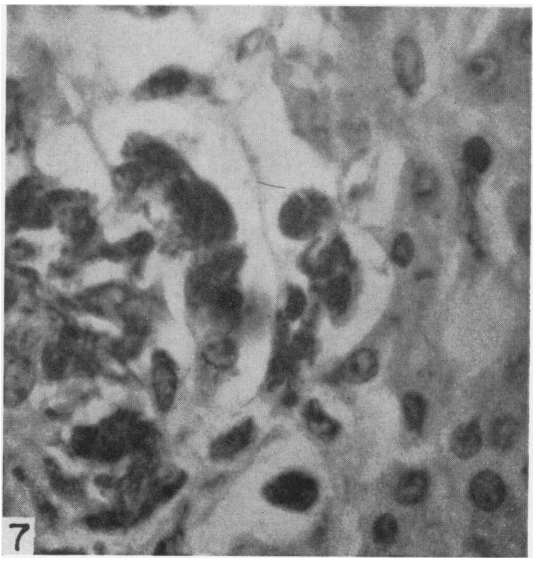


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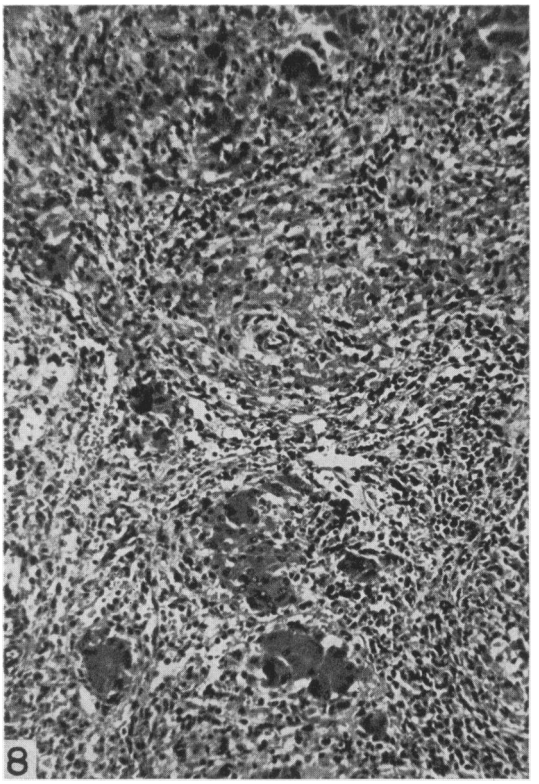
PLATE 3

FIG. 8. Spleen showing multinucleated giant cells and concentrically arranged epithelioid cells about the central arteries of replaced malpighian corpuscles. Hematoxylin and eosin stain. × 140.

FIG. 9. Lymph node showing large mononuclear macrophages and giant cells. Some giant cells have clear, central cytoplasm surrounded by a foamy, peripheral zone. The displacement of normal lymphoid structure is illustrated. Hematoxylin and eosin stain. × 140.

FIG. 10. Spleen showing massive, acid-fast, intracellular parasitism of both large mononuclear macrophages and multinucleated giant cells. The absence of organisms in the interstices is a characteristic feature. Ziehl-Neelsen's stain. × 1400.

FIG. 11. Lymph node showing two of the giant cells seen in Figure 9. The clear, central zone results from absence of organisms, while in the periphery of the cells they are found in a radiating configuration. The extent of intracellular parasitism by acid-fast organisms is evident. Ziehl-Neelsen's stain. × 750.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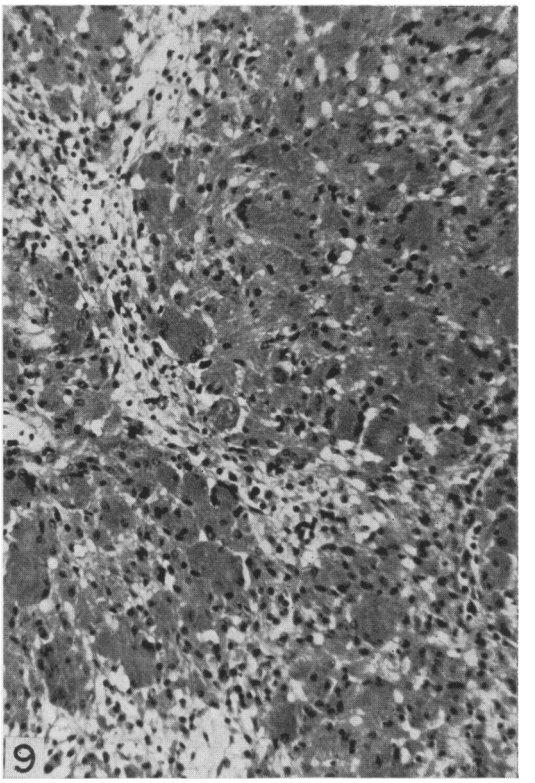
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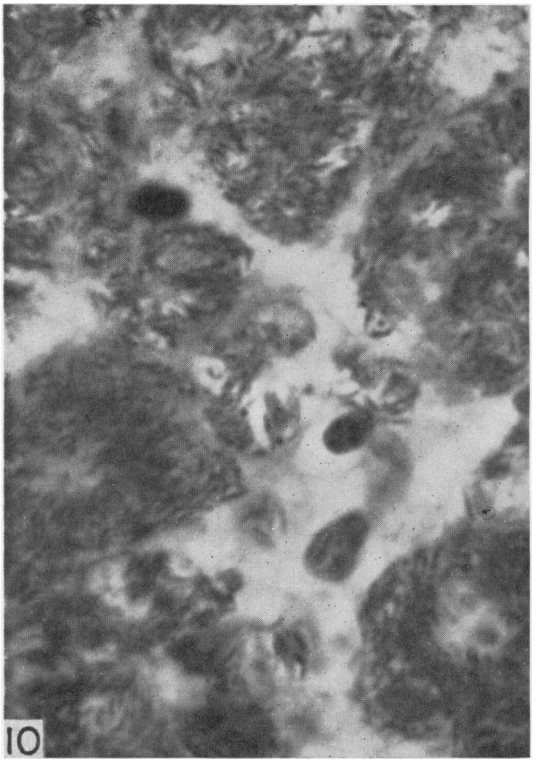
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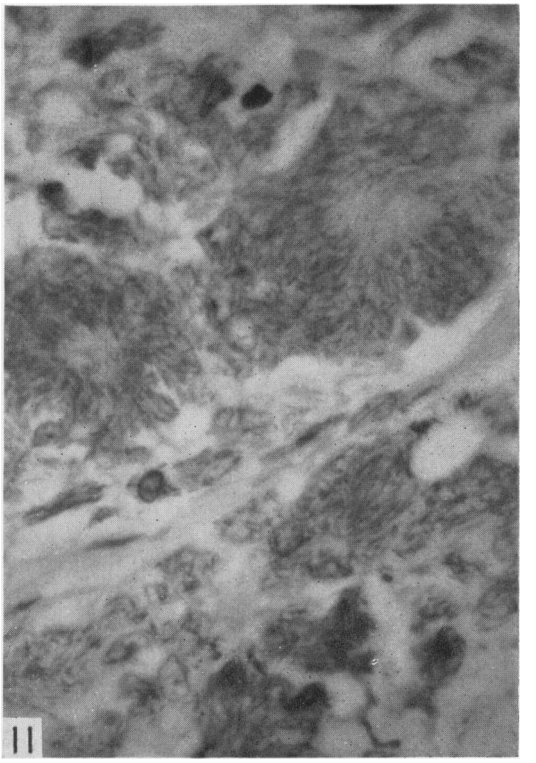
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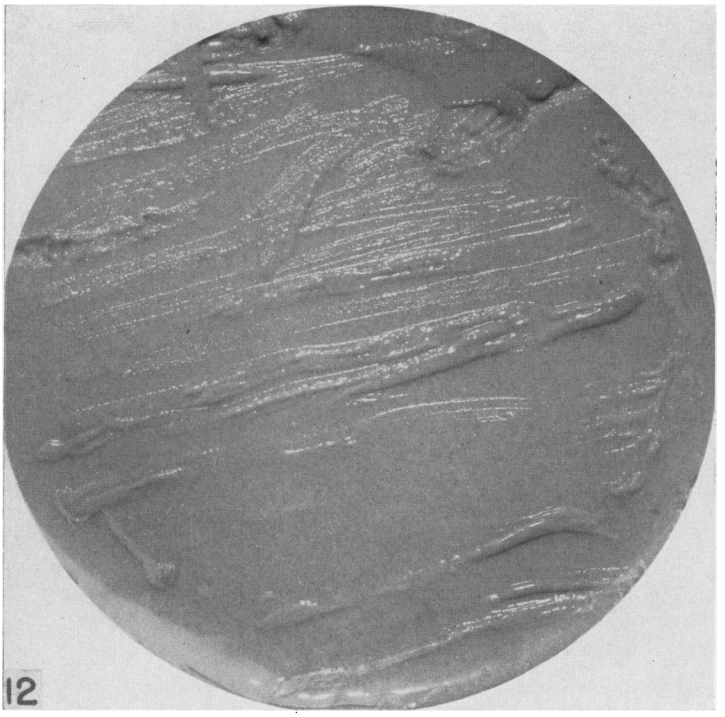
PLATE 4

FIG. 12. A 10-day-old streaked plate of the causative organism on glycerin-egg medium, showing the smooth, confluent, moist growth without aerial mycelia.

FIG. 13. A 6-months-old culture on Sabouraud's slant illustrates the vermiform contour of the growth.

FIG. 14. A magnified view of a 12-day-old streak culture on Sabouraud's agar to show the details of colony growth. The individual colonies tend to coalesce and produce the smooth growth seen grossly in Figure 12. × 200.

FIG. 15. A stained smear of the organism transferred by loop from Sabouraud's agar slant (Fig. 13). The pleomorphic forms result from the fragmented hyphae. Gram's stain. × 1350.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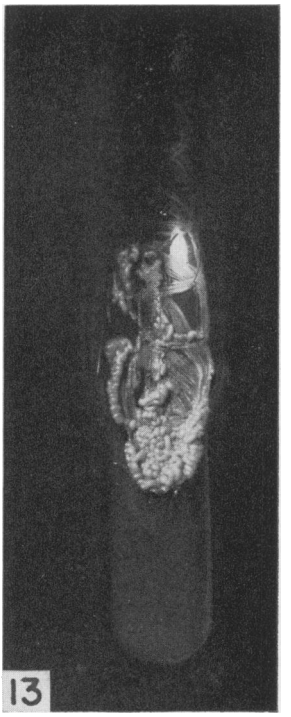
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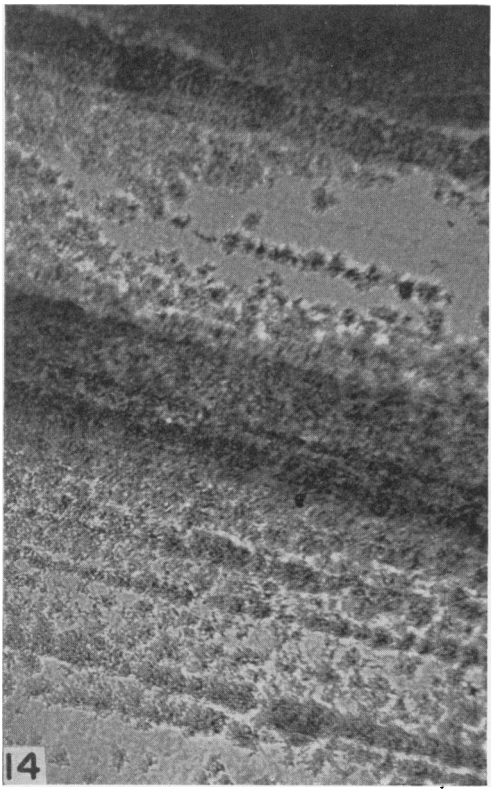
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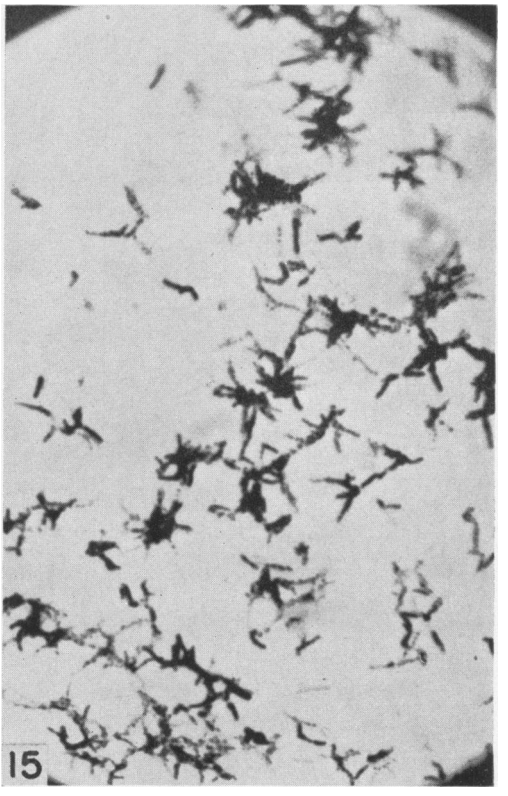
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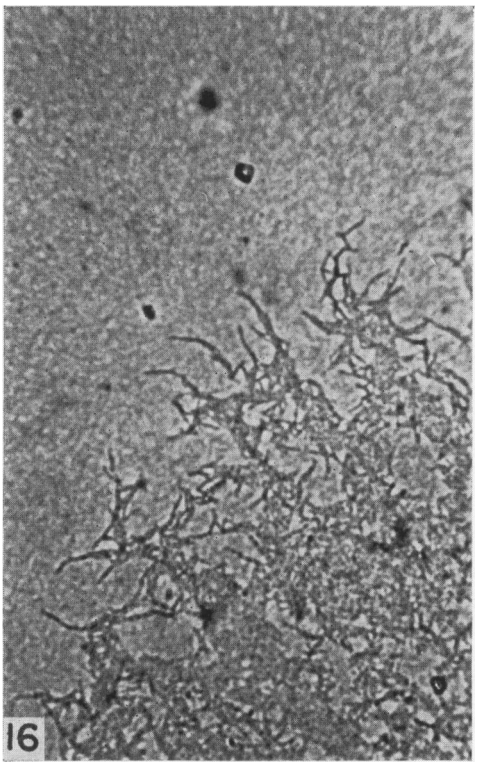
PLATE 5

FIG. 16. A photomicrograph of the unstained organism growing on Sabouraud's agar in a Van Tieghem cell mount, showing mycelial growth with true branching. × 800.

FIG. 17. A stained smear of the organism from a 10-day-old litmus milk culture, with preservation of true branching and regularly spaced, spherical to ovoid bodies identified as arthrospores. Kinyoun's acid-fast stain. × 1700.

FIG. 18. Another view of the smear of the organisms from the 10-day-old litmus milk culture (Fig. 17), showing matting of hyphae. At the periphery of the mass may be seen the large pleomorphic, irregularly arranged, nonacid-fast bodies termed granules. Kinyoun's acid-fast stain. × 1350.

FIG. 19. A high-power view of a lymph node of a rat to show the scattered acid-fast organisms in a collection of large mononuclear macrophages. At left is a "Y" form (arrow). Ziehl-Neelsen's stain. × 1700.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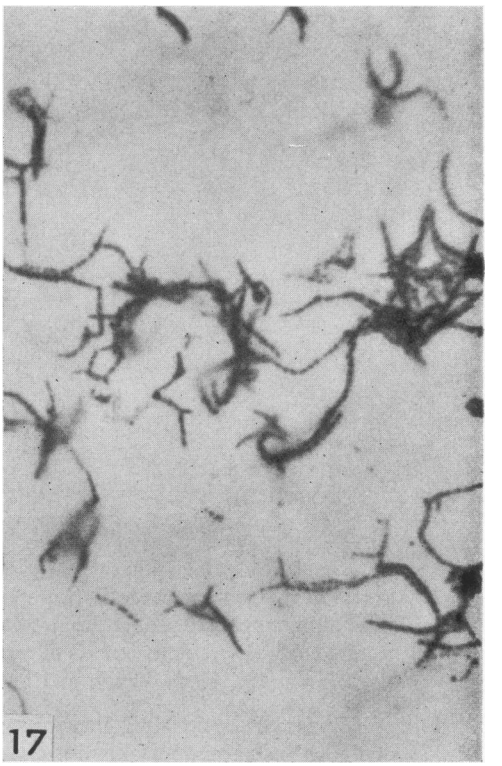
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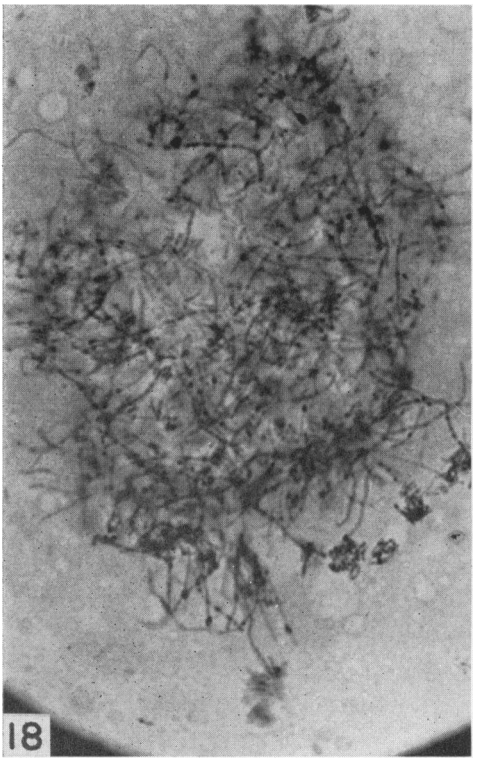
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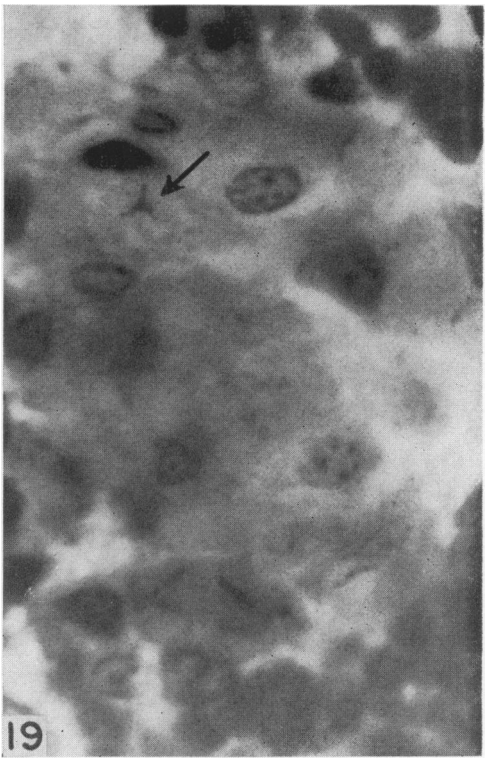
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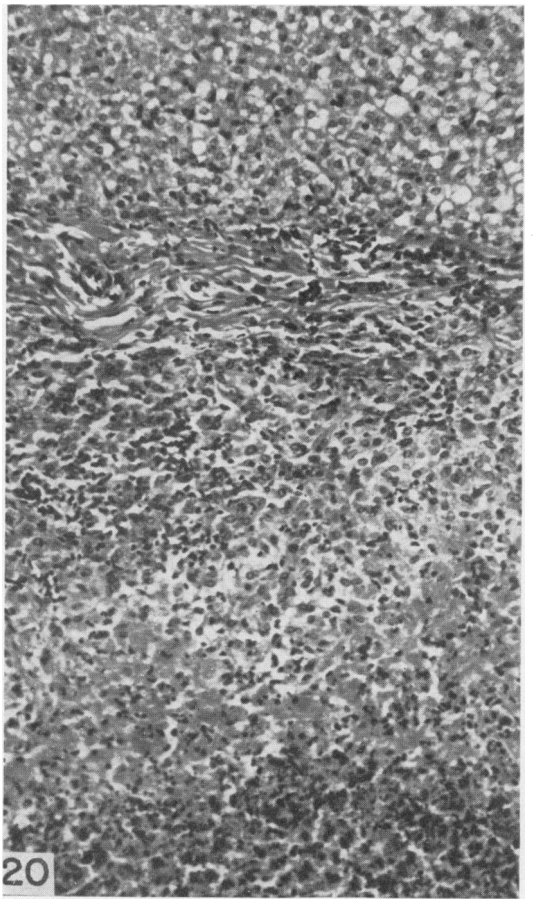
PLATE 6

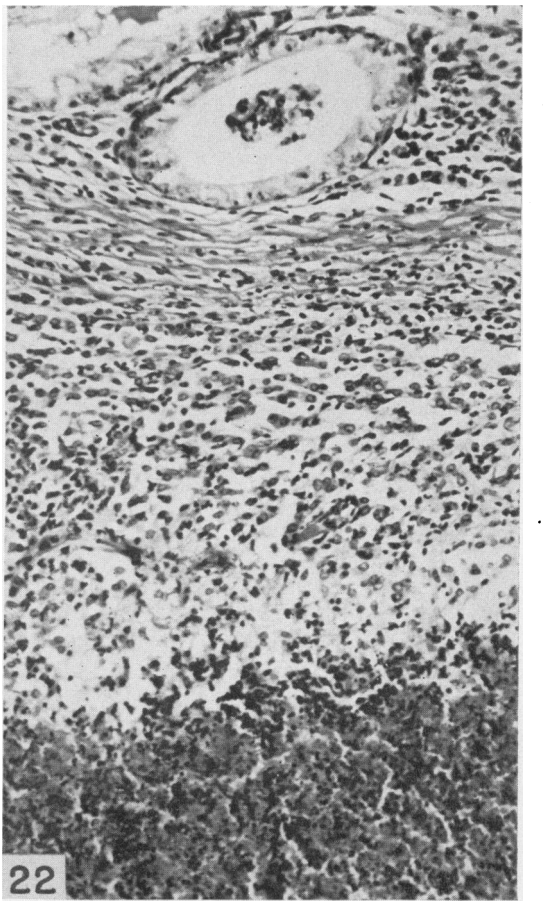
FIG. 20. Liver of a guinea-pig 4 weeks after subcutaneous injection of viable organisms. In the zone of necrosis (lower), organisms were found in clumps; in the zone of macrophagic proliferation (center), a few isolated intracellular parasites were found. There is a zone of fibrosis separating the lesion from normal liver tissue (above). Hematoxylin and eosin stain. × 150.

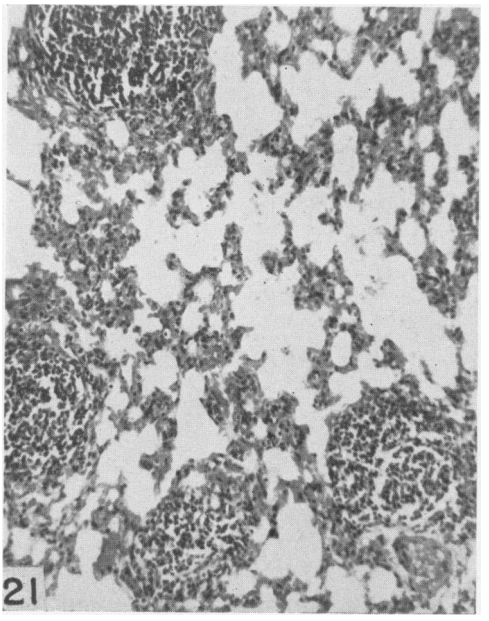
FIG. 21. Lungs of a guinea-pig showing enlargement of lymphoid follicles. In only one such instance were organisms stained within these follicles. Hematoxylin and eosin stain. × 150.

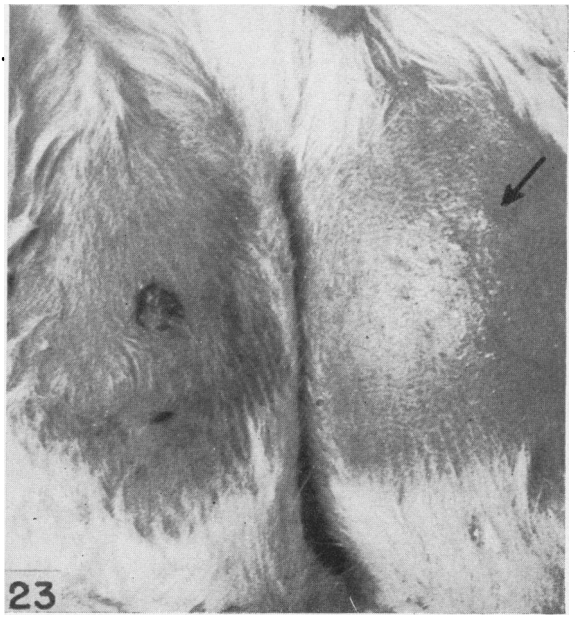
FIG. 22. Epididymis of a guinea-pig with necrosis in a central zone surrounded by macrophages as in Figure 20. Many acid-fast organisms were found in clumps in the necrotic tissue. They are fewer in number among the macrophages. Hematoxylin and eosin stain. × 200.

FIG. 23. Two mice showing sites of inoculation after 24 hours (right) and 7 days (left). The 24-hour lesion is a small papule seen at the arrow. The lesion after 7 days is a large ulcer.









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