AN UNUSUAL ACID-FAST INFECTION OF THE KNEE WITH SUBCUTANEOUS, ABSCESS-LIKE LESIONS OF THE GLUTEAL REGION

Report of a Case with a Study of the Organism, *Mycobacterium abscessus*, n. sp.*

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Human infection with acid-fast bacilli other than the usual human, bovine and avian tubercle bacilli and the lepra bacillus is not common. In the past, the tendency to diagnose as tuberculosis all lesions in which there were demonstrable acid-fast bacilli and/or a tissue picture suggesting tuberculosis was very strong. In recent years, however, it has been demonstrated that acid-fast bacilli, clearly not the commonly accepted tubercle bacilli, may produce human infection. Such lesions present to the pathologist a problem in diagnosis since they resemble tuberculosis histologically. Bacteriologically, however, the organisms isolated from such cases vary distinctly from the tubercle bacilli, culturally, although simulating them closely in tissue. In addition, lesions produced by these 'pseudo-tubercle' bacilli usually follow trauma, remain fairly well localized, show little or no tendency to produce progressive disease, and invariably clear up in a period of approximately six months to two years with or without treatment.

We are reporting in this paper such a case of human infection with an unusual history of 48 years from the time the traumatic infection was contracted to the time diagnosis was finally established. Because the causative agent is considered to be a new and hitherto undescribed species of mycobacterium, the reporting of the case and a study of the organism is deemed advisable.

REPORT OF CASE

Mrs. I. D. C., a 63 year old white woman was admitted to Barnes Hospital on January 29, 1950 with a chief complaint of stiffness, pain, swelling and limitation of motion of the left knee for the past six to eight months.

The past medical history revealed that at the age of 14 years the patient had traumatized and dislocated the left patella while on a farm. She was treated by her local physician and experienced only stiffness and vague pains, but no difficulty in locomotion. The vague pains persisted intermittently and in June, 1949 the pain and swelling became accentuated. She treated herself with heat, cold, liniments, bandage and rest. Improvement was transitory and in January, 1950 she came to the Washington University Clinics and was admitted for corrective surgery. A roentgenogram of the left knee revealed hypertrophic osteoarthritis and a soft tissue mass of the antero-lateral portion of the knee joint. (Fig. 1)

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Physical examination showed a short, slightly obese woman who appeared unusually well preserved. She was intelligent and cooperative. Her skin was unusually clear and free from blemishes. An evaluation of all systems and organs was well within normal limits. The blood pressure was 134/86 mm. Hg., pulse 72, respiration 18 and temperature 37°C.

Laboratory studies on admission showed the blood count to be as follows: hemoglobin, 11.0 gm. %; red cells, 3,930,000; white cells, 6,500; segmented forms, 69%; lymphocytes, 24%; monocytes, 3%; band forms, 2%; eosinophiles, 2%; basophiles, 0; myelocytes, 0. The blood Kahn reaction was negative. The urine had a specific gravity of 1.021; pH 4.5; no albumin, sugar or acetone. There were no casts or crystals and one or two white blood cells per field. The stool was black and guaiac negative.

On January 30, 1950 a patellectomy and synovectomy were performed. The pathologist reported normal bone for the patella except for fibrous inflammatory tissue beneath the

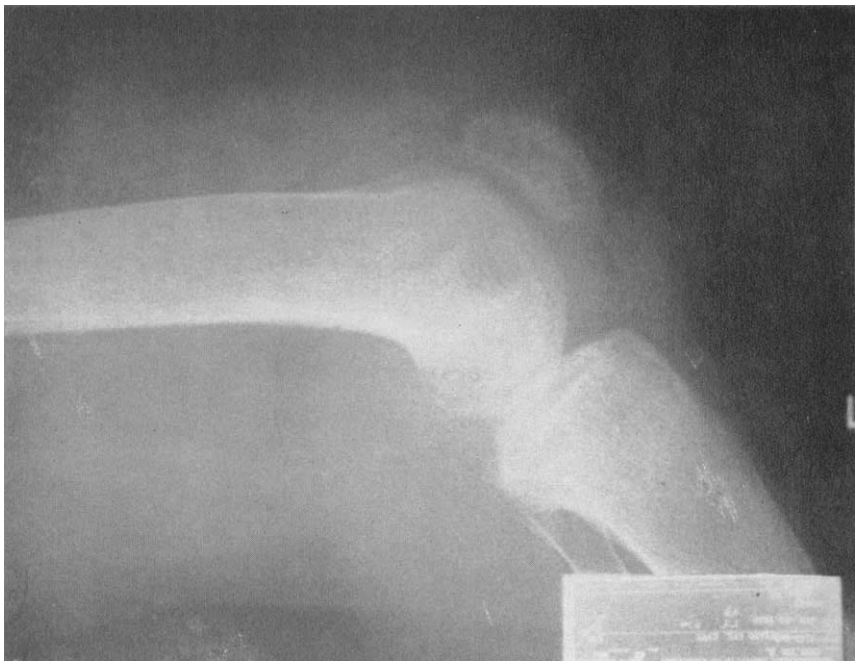

FIG. 1. Roentgenogram of left knee showing hypertrophic osteoarthritis and soft tissue mass of antero-lateral portion of knee joint.

cartilage. The synovium showed a granulomatous reaction with occasional giant cells and surrounded by fusiform cells. Sections of the synovium stained for acid-fast bacilli showed many organisms and a diagnosis of tuberculosis was made.

Cultures made of synovial tissue developed an atypical acid-fast organism. A guinea pig was inoculated but was later reported as negative.

On February 9, 1952 an arthrodesis of the knee was performed. Sections of the tibia and femoral condyles were examined by the pathologist. He observed granulation tissue, necrosis with amorphous material and many giant cells, and made a diagnosis of tuberculosis. The patient was treated with penicillin and streptomycin injections from January through April while in the hospital. She left the hospital to return in June.

In June, 1950, when the cast on the knee was removed, it was noticed that the patient had some swellings in the left buttock. These were not tender but became more prominent

while she was in a sitting position. She was told by the surgeons in the clinic that these were probably a result of the streptomycin injections. Two weeks later the areas opened and drained spontaneously. Hot packs and sitz baths were not effective and on July 6, 1950 she was admitted to the hospital. At that time similar indurations were noted in the opposite buttock. On July 22, 1950 the lesions in both buttocks were opened, excised and drained. The lesions in the left buttock were described as large draining points of a common communicating gluteal abscess. The involved area extended down to the paramuscular fascia of the gluteus maximus muscle. The same was true of the lesion in the right buttock. Pathologically, the section of excised tissue revealed acute and chronic inflammation, proliferation of new blood vessels and focal areas of necrosis. Acid-fast organisms were not found in the tissue and the diagnosis was 'chronic inflammation.'

Pus obtained from the draining sinuses was cultured on Petragnani medium on three different occasions and acid-fast organisms, similar to those isolated from the knee, were cultured and described as atypical tubercle bacilli. A guinea pig was injected with some of the cultured organisms but had no lesions when examined six weeks later. The operative sites in the buttocks did not heal completely and multiple draining sinuses developed, some in the excisional areas. Fourteen such sinuses (including unruptured nodules) were counted in the left buttock and eight in the right buttock. The number varied from time to time as new lesions developed.

A roentgenogram of the pelvis showed no pelvic abnormality. A roentgenogram of the chest showed clear lung fields. Material aspirated from the sternal bone marrow and cultured did not develop organisms. On September 19, 1950, first strength P.P.D. injected intradermally resulted in a positive test with redness, swelling and induration 2 cm. in diameter. The histoplasmin test was negative. All other clinical laboratory data were within normal limits and did not contribute any information towards a diagnosis other than what has been reported.

On suspicion that the acid-fast organism might be an acid-fast Nocardia, material from an unruptured nodule was planted on Sabouraud's glucose agar. The same organism described as an atypical acid-fast tubercle bacillus or as a saprophytic mycobacterium grew out readily.

The lesions slowly cleared and approximately 18 months after the time they were first noticed, the lesions of the buttocks had disappeared.

PATHOLOGY

Pathology of synovium. Grossly there were numerous flat bodies attached to the synovium which varied in size from a few millimeters to two centimeters in diameter (Fig. 2A). These were loosely attached and they were thick towards the center and tapered off to a fine edge. They were resilient in consistency and appeared to be more fibrinous than cartilaginous. The synovium was quite thick.

Sections of the synovium showed thickening of the wall with several fairly round areas of granulation tissue in which there was an occasional giant cell surrounded by fusiform cells (Fig. 2C). There were many large amorphous, pink-staining areas (fibrin) loosely attached to the wall of the synovium. In some areas bordering these fibrin bodies there was a palisading arrangement of cells. The section of meniscus did not show anything unusual. Sections of the patella showed normal bone. Some sections showed fibrous, inflammatory tissue beneath the cartilage. Sections of the synovium stained for acid-fast bacilli showed many organisms in the amorphous attachments to the synovium.

Pathology of femur and tibia. Grossly there was no evidence of bone destruction. In the tibial articular surface there was an area which was soft and mushy.

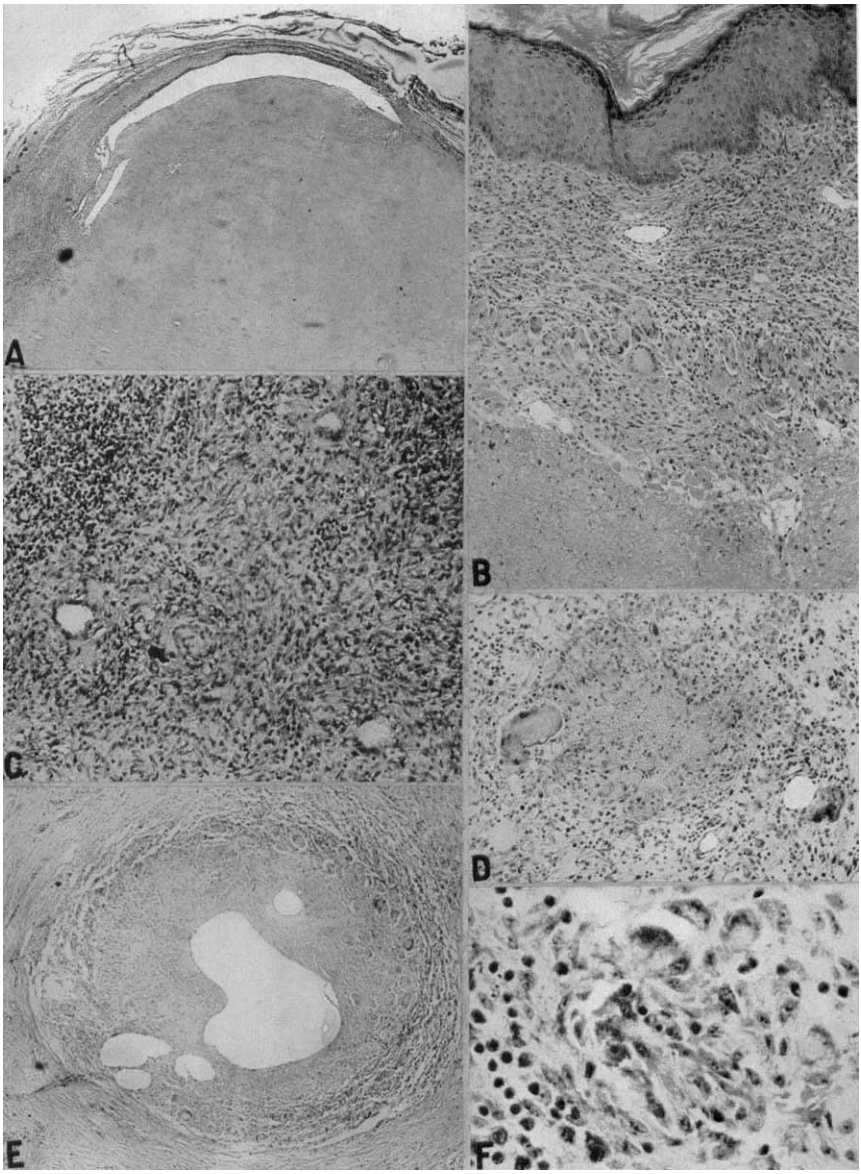

FIG. 2. Sections of human tissue. A. Portion of amorphous body attached to synovium. Hematoxylin and Eosin. × 70; B. Lesion of gluteal region showing inflammatory response with zone of caseation necrosis. H. & E. × 135; C. Soft tissue of knee lesion with tuberculoid response. H. & E. × 175; D. Tuberculous-type nodule in cutis of gluteal lesion. H. & E. × 145; E. Partial obliterative endarteritis in subcutis of gluteal lesion. Ziehl-Neelsen. × 65; F. Small noncaseating tubercle in gluteal lesion. Z.-N. × 660. All magnifications before one-half reduction.

Sections of the tibia showed viable cancellous bone in which there was a well circumscribed area of granulation tissue which had infiltrated the spaces. There

was a central area of necrosis containing amorphous material. Many giant cells were present.

Sections of the lateral and medial femoral condyles showed cancellous bone with granulation tissue at the junction of the bone with the cartilage. Many giant cells were present along the cartilage-bone junction.

Pathology of skin and subcutaneous tissue. Tissue was excised from the lesion of the buttocks. Externally the tissue showed no unusual abnormalities. On section, however, there was revealed a zone of opaque yellowish irregularity in the cutis, which replaced most of the cutis and extended deep into the subcutaneous fat.

Microscopically, the epidermis appeared to be normal except for a slight flattening in the area extending above the surface of the opaque tissue noted in the gross. In the lower cutis there was the beginning of a large zone of caseous necrosis of the tissue that extended down into the subcutaneous fat. Surrounding this area there was a zone of fibrous tissue in which there was evidence of granulation with the proliferation of new blood vessels. In the tissue surrounding the central caseous zone (and extending into the upper cutis) there were numerous giant cells, epithelioid cells and lymphocytes (Fig. 2B). Some giant cells occurred in definite small tubercles (Fig. 2F) associated with epithelioid cells and lymphocytes, while others were present around small areas of caseous necrosis unquestionably representing necrotic tubercles (Fig. 2D). Tissue stained for bacteria and for acid-fast bacilli failed to reveal organisms and the diagnosis was considered to be chronic inflammation with caseation.

An evaluation of the histopathology of this lesion seems to indicate rather clearly that the lesion began in the subcutaneous fat and extended upwards to the epidermis. Since the organisms isolated from the lesions of the buttocks were identical with those recovered from the knee lesion, it is further apparent that the bacilli had spread by way of the blood stream to the gluteal region. The tissue reaction emphasizes this point. This hematogenous spread was undoubtedly caused by the surgical trauma to the knee. Because of the injection of streptomycin and penicillin, an area of lowered resistance, a *locus minoris resistentiae*, was established allowing the bacilli to localize and grow in a traumatized area and thus develop the lesion described above.

REVIEW OF LITERATURE

There are few reports in the literature on human infection caused by acid-fast bacteria other than the usual human, bovine and avian tubercle bacilli and the lepra bacillus. In 1918, Cobbett (1) described an acid-fast bacillus which produced intractable pustules and deep subcutaneous abscesses covering the back, buttocks and thighs of a 24 year old soldier who had been wounded in the foot. The eruption developed about a month after he had been exposed in the water for one hour following the sinking of a boat on which he was being transferred to England.

Primary cultures developed only a few colonies from the pus. These were large and cream-colored. The bacilli were long, slender, slightly curved at first, showing an irregular segmentation. On subculture, the bacilli were shorter and plumper and stained badly. The organism grew well at 17°, 22° and 37°C. In glycerin broth, a superficial film was formed which remained flat and thin, never wrinkled and did not climb up the sides of the tube. The fluid remained clear with a dense deposit on the bottom of the tube.

In the animal inoculations, 20 mg. bacilli injected intraperitoneally in a guinea pig showed a caseous nodule in the omentum one month later. Ten mg. injected subcutaneously in a rabbit resulted in a soft mass which persisted four months. Mice were given a 10 mg. dose intraperitoneally. One mouse died in 12 days and showed numerous small abscesses in the kidney and one caseous area in the liver.

In 1931, Beaven and Bayne-Jones (2) reported a case of extensive pulmonary disease resembling tuberculosis in a child. At the age of 11 months the right lung was found to be consolidated. One month later an acid-fast organism was isolated from the pleural fluid.

The organism was aerobic, giving rapid growth, producing colonies in 24 to 48 hours and abundant development after seven days. The bacillus grew well at 22° but better at 37°C. On glycerin egg medium the growth in 24 hours was thin, glistening and transparent. This became pearl gray, moist and gelatinous with a yellowish cast. In older cultures the yellow pigment became tinged with red. On solid medium there developed rough and smooth colonies. In Douglas broth with 5% glycerin, inocula from rough colonies produced a white uneven pellicle with broken flaky masses. The pellicle mounted the sides of the flask evenly and abundantly. The broth remained clear with a granular growth at the bottom. The pellicle became thick, dry, did not break up or sink to the bottom and remained white. Inocula from smooth cultures produced clouding of the broth. The pellicle began as a thin dull gray web, spreading over the surface of the broth. In older cultures the organism mounted the sides unevenly and the smooth creamy surface became a dense, grayish-yellow film with folded ridges.

The bacteria from rough colonies measured 0.4 and 1.2 by 1.2 and 0.3 microns. In smooth colonies the bacilli measured 10 and 2 by 1.2 and 0.2 microns. These were maximal and minimal measurements. In young cultures the bacilli stained diffusely with the Ziehl-Neelsen stain and in older cultures they were beaded and granular. The bacilli resisted decolorization for 45 minutes. In a saline suspension the organism was killed at 60°C., in 10 minutes.

Using heavy suspensions of bacilli, inoculations were made subcutaneously in the rabbit and guinea pigs. The animals were killed 70 days later. The rabbit showed a caseous lymph node and a guinea pig had developed caseous lymph nodes in the inguinal region on the side injected. The lesions appeared rapidly, forming nodules with a thick, mucoid, caseous or purulent material. The optimum time for the reaction was 8 to 10 days and the nodules disappeared usually after two weeks. Intraperitoneal injection of 0.5 cc. suspension showed 9 to 10 days later that the omentum was infiltrated. There was a fibrous perisplenitis with invasion of the liver capsule and some areas of the liver substance.

Branch (3) studied the strains of Cobbett ("Cook" strain) and that of Beaven and Bayne-Jones ("Ryan" strain) and concluded that the "Ryan" strain resembled the "Cook" strain in culture. Both grew at 17°, 22°, and 37°C., not at 42°C. Both grew rapidly and on egg and glycerin medium the colonies were large, dry and did not emulsify readily. In broth, the "Cook" strain formed a pellicle which sank easily whereas the "Ryan" strain formed a thick pellicle which did not sink easily. The "Ryan" strain on egg formed a yellow pigment while the "Cook" strain did not.

In 1944, Gellerstedt (4) reported seven cases (4 children and 3 adults) with skin nodules, boils or limited swellings which ulcerated and were caused by atypical acid-fast bacilli. In six patients the lesions were located peripherally on the extremities (elbows and knees) and in one patient the cheek was involved. In six patients there was regional adenitis. The skin ulcers were round, raised, varying in size from a shilling to that of the palm, with a discharging base which was either necrotic or covered with granulation. The surrounding skin was reddish or cyanotic and the edges sometimes undermined. The lesion progressed slowly, but did not extend beyond the primary ulcer and the regional nodes. The lesions were benign in their course and did not recur. In five patients the duration was between three and six months and in the other two it was 10 months to one year.

The histologic diagnosis was granuloma with atypical acid-fast bacilli. The skin showed a tuberculoid response with giant cells, caseous or purulent necrosis with a purulent exuda-

tion or small abscesses in the granulation tissue. There was also a diffuse dispersal of epithelioid granulomas without a 'true tuberculous picture.' The tissue further showed vasculitic changes with lymphocytes, plasma cells and non-specific granulation tissue. Numerous bacilli were found in all the cases. These were acid-fast, short, thick rods about one-half to one-third the size of true tubercle bacilli, pointed or tapering, club-like, coccoid, granular or vacuolated in form. They were in bundles, often phagocytized in the form of compact balls similar to globi. The bacilli were seen only in a small area of the granuloma usually in epithelioid granulation tissue, but also in the caseous or suppurative zones. Unfortunately no cultures were obtained from any of the seven patients. Gellerstedt believed the organism to be somewhat similar to the one which produces skin lesions in tuberculin-reacting cattle. He further considered them to belong to a group of approximately 140 cases described in the 10 previous years as skin swellings which were diagnosed histologically as granuloma of unknown etiology.

In the same year, Englund and Wahlgren (5) described two instances of post traumatic inflammatory dermatoses (one involving the wrist and the other the elbow) in young men, accompanied by regional lymphadenitis. The lesions consisted of deep cutaneous infiltrations in areas of trauma with a subsequent break down of the skin. Histologically, the cutis and subcutis in the early stages showed inflammation and granulation tissue with many epithelioid cells but no tuberculoid structures. There were many acid-fast bacilli which were shorter and thicker than the tubercle bacilli, appearing often in bundles. In the later stages the granulation tissue assumed a tuberculoid appearance with epithelioid cells and caseous necrosis. At this stage acid-fast bacilli could no longer be observed in the tissue. Cultivation of the organism and transfer of material by inoculation into rabbits, guinea pigs and mice gave negative results. In the main, these cases agree with those described by Gellerstedt.

In 1948, MacCallum, Tolhurst, Buckle and Sissons (6) reported a series of lesions occurring either on the skin or forearm of seven patients. All the lesions were ulcers and each teemed with acid-fast bacilli. Histologically, no tubercles, giant cells or caseation were seen in any of the sections. The bacilli in most tissue sections were engulfed by phagocytes and only a few were free in the tissue.

The organism in tissue resembled the human tubercle bacillus, measuring 3 to 6 microns in length, varying from 0.75 to 1.3 microns and approximately 0.2 to 0.35 microns in width. The bacilli had parallel sides, rounded ends and were often curved. Branching was seen occasionally. Within mononuclears and polymorphonuclear leucocytes the organism attained a size of 1.3 by 7 microns. The organism was strongly acid-fast, gram positive and displayed round or oval granules or beads in the center or at the ends. The organism grew well at 30° or 33°C. On Petragnani medium growth was obtained in 30 days. The colonies were tiny, transparent, smooth, dome-shaped, becoming convex or umbilicate. Primary growth was not obtained on nutrient agar or nutrient agar plus 5% glycerin. Growth was absent or poor in six to eight weeks on blood agar, Dorset's egg medium or Loeffler's serum agar. The organism was killed after 30 minutes at 60°C. The authors considered the organism to be a new species and named it *Mycobacterium ulcerans*.

In experimental animal inoculations, the epididymis of rats was found to be the preferred site. Dissemination possibly occurred through the blood stream and lymph channels, spreading in the subcutaneous tissue and then penetrating to the skin surface. Ulcers developed on the tail and limbs of both male and female rats. Ulcers of the skin developed after subcutaneous inoculation and showed signs of localized spread through the subcutaneous tissue. A similar spread was noted in the human tissue. In addition lesions in the rat commonly showed thrombosis and essential obliteration of small blood vessels in the necrotic area. This was also noted in the tissue of the patient reported in this paper and in the chick inoculations.

In 1951, there appeared in the program of the International Congress of Clinical Pathologists a report by Herlitz, Linell and Norden (7) on the observation of a number of cutaneous lesions, chiefly on the elbow, caused by acid-fast bacilli. This work was fully reported in

Nature in the same year by Norden and Linell (8). The authors reported about 70 cases of a chronic, papulous, cutaneous disease from Orebro, Sweden. The patients reported that they had scratched their elbows in a swimming pool two to three weeks previously. The wound healed spontaneously, but two to three weeks later a papule, the size of a bean, developed in the scar. This lesion ulcerated and discharged a scanty thick fluid and eventually healed after six months to two years leaving a soft scar.

Histologically, the tissue from 25 patients showed a granulomatous response with a diffuse proliferation of epithelioid cells and scattered giant cells, and occasionally with many lymphocytes. A tuberculoid structure with 'central fibrinoid necrosis' was noted in some sections. In only one case were organisms observed with the Ziehl-Neelsen stain.

In three out of 20 cases studied bacteriologically, an organism simulating the tubercle bacillus was isolated on Lowenstein's medium in eight to 10 days when grown at 31°C. Slow growth was obtained at 22°C., in three weeks, and no growth at 37°C. The colonies were yellowish-white, flat, with a central nipple. Growth was good on glycerin agar and poor when glycerin was omitted. An organism showing the same characteristics was isolated from the water and walls of the suspected swimming pool. Tissue changes similar to those found in the patients were obtained by rubbing cement from the pool wall into the skin of a rabbit. Cultures from the rabbit skin yielded a growth of bacilli similar to those described.

Injection by various routes in guinea pigs, rats, rabbits, and chickens did not produce progressive lesions. In the guinea pigs, localized abscesses in the scrotum developed. Percutaneous inoculations resulted in papulous lesions similar to those in humans. Intraperitoneal and intravenous injections of bacilli in rabbits developed granulomatous inflammation and caseous necrosis in the scrotum. Intraperitoneal injections in white mice resulted in abscesses and indurations on the tail, paws and scrotum with widespread internal lesions in the lungs, which showed caseous patches. Some of the white mice died. Rats showed no signs of disease, but microscopically there were found numerous tuberculoid structures in the omentum and hilar lymph nodes.

In some respects the animal inoculations tend to simulate those reported by MacCallum, Tolhurst, Buckle and Sissons.

Further cases resulting from trauma in swimming pools were reported by Cleveland (9) and also by Hellerstrom (10). Cleveland presented four patients with granulomatous lesions of the skin (two of the chin, one of the left little finger and one of the bridge of the nose) which developed after abrasion from contact with material submerged in a swimming pool. The clinical and microscopic appearances of the lesions strongly suggested tuberculosis cutis. Acid-fast bacilli were not seen in the tissue.

Hellerstrom reported six patients with the lesions occurring on the nose following trauma in a swimming pool. The clinical picture was that of lupus vulgaris. In no case was the organism cultured and in only one were acid-fast bacilli seen in tissue. Histologically, the lesion showed tubercles, epithelioid cells in groups, giant cells with some showing caseation necrosis. He believed these cases to be primary inoculation lupus vulgaris.

In a communication, following the paper of Cleveland (Acta Dermato-Venerol. **31:** 152, 1951) Hellerstrom stated that tubercle bacilli were found in one of the swimming pools by inoculating water sediment in guinea pigs. Bacilli obtained from guinea pig material were grown on special mediums.

Swimming pool lesions of a similar type have been reported from Washington and Oregon at the Pacific Northwest Dermatological Society held in Seattle, October 19, 1950.

In 1951 also, Pollak and Buhler, in the Proceedings of the American Association of Pathologists and Bacteriologists (11) reported two fatal cases caused by atypical acid-fast bacilli. The first was a 21 year old white man who at necropsy revealed large mesenteric nodes, the structure of which was replaced by partially calcified, caseous and purulent masses. There were caseous masses in the spleen and lungs. There were found large acid-fast bacilli, necrosis and few giant cells.

The second case was that of a four week old infant with cyanosis and dyspnea, developed two weeks previously. The baby was admitted to the hospital in a moribund state with pneu-

monia and died 24 hours later. On post-mortem examination, there was found a widespread 'bronchopneumonic consolidation.' Microscopic examination revealed large, elongated acid-fast bacilli in inflammatory foci. Similar lesions were found in the hilar lymph nodes. Small miliary tubercles were seen in the liver and spleen with numerous acid-fast bacilli.

The organism was large, beaded and strongly acid-fast and it occurred in packets and clumps. It grew easily on Petragnani's medium, developing yellow colonies. In guinea pigs it produced a mild, self-limited disease and was mildly pathogenic for mice.

These are perhaps only a few of the reported instances of lesions caused by atypical acid-fast bacilli. The papers reviewed here, for the most part, refer to cutaneous manifestations and as such have some bearing on the case being reported in this paper.

BACTERIOLOGY

In sections of the synovium of the case reported here the acid-fast bacilli appeared singly or in clumps either freely distributed in the amorphous, flat bodies loosely attached to the synovium, or associated with groups of leucocytes (Fig. 3A). The bacilli were strongly acid-fast varying considerably in size and shape, being either short and thick or long and thin, straight or curved (fusiform), with rounded or slightly pointed ends. Coccoid forms could be seen occasionally. The acid-fast bacilli appeared either as solid forms or for the most part as beaded or perhaps vacuolated structures. Branching forms were not observed. The bacilli measured approximately 0.2 to 0.5 by 2 to 6 microns for the bacillary forms some attaining a length of 7 microns. Many bacilli measured 5 to 6 microns in length while many others only measured 3 to 4 microns. Short forms were also noted, these measured 0.3 to 0.5 by 2 to 3 microns. Coccoid forms commonly measured 0.5 microns in diameter.

In pus obtained from the buttocks lesions the bacilli were also strongly acid-fast and were seen chiefly in clumps, but also as isolated forms, measuring the same as those seen in tissue, although some forms appeared thinner, approximately 0.2 microns in diameter (Fig. 3D). In general, however, the measurements approximated closely those of the bacilli, short and long forms and coccoid structures, seen in tissue.

Cultures made at Barnes Hospital from the synovial tissue developed well in a few days on Petragnani medium in the incubator. Similar organisms were cultured on the same medium in the same way from the lesions of the buttocks.

On suspicion that the organism might be an acid-fast Nocardia, chiefly because of its rapidity and abundance of growth, and because it was not a characteristic tubercle bacillus, mycologic cultures were made. The primary isolation was made on Sabouraud's glucose agar. Growth at room temperature was slow, colonies becoming visible on the fourth day and well developed on the seventh day. In the incubator, 37.5°C., primary isolation on Sabouraud's agar developed well after four days. The growth consisted of numerous, small colonies measuring 0.5 to 2 mm. in diameter. These were chiefly smooth, rounded (hemispherical), some showing mammiliform characteristics. The colonies were light cream in color, becoming dark cream to buff with age.

In order to check the difference in growth at room temperature and at 37.5°C., subcultures were made on Sabouraud's glucose agar, dextrose agar plus 5% glycerin, nutrient broth and dextrose broth plus 5% glycerin. *On the third day*

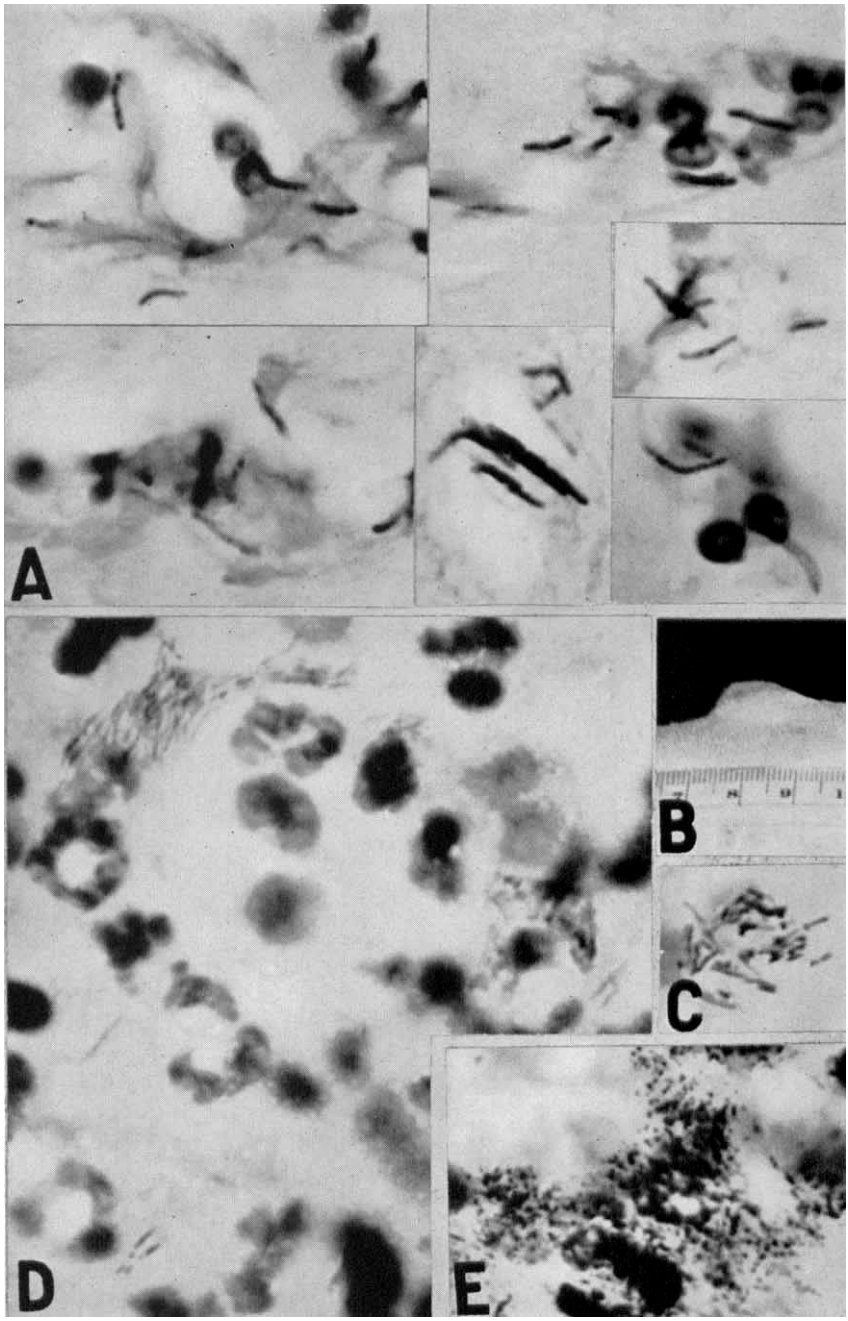

FIG. 3. A. Composite photomicrograph showing various forms of bacilli in synovial tissue. Ziehl-Neelsen. × 2240; B. Intracutaneous nodule in rabbit skin of abdominal wall nine days after injection; C. Smear of pus from intracutaneous rabbit nodule showing granular bacilli. Z.-N. × 2160; D. Smear from lesion of gluteal region showing clumps and single bacilli. Z.-N. × 2160; E. Smear of bacilli from intracutaneous rabbit nodule showing Gram positive granules. × 2160.

there was good growth at 37.5°C., on the agar substrates, that on dextrose-glycerin being dull, while that on Sabouraud's glucose was shiny. In the dextrose-glycerin broth a thin veil-like cloud could be seen in the medium, whereas in the nutrient broth there was apparent a fine suspension. Cultures at room temperature were too poorly developed for description.

On the fifth day at 37.5°C., growth was well developed on the agar mediums. On Sabouraud's agar the growth was smooth, shiny and moist. On dextrose-glycerin agar growth was dull, rough, dry-appearing with a tendency to flake off with the inoculating needle. Nutrient broth showed a coarser suspension while dextrose-glycerin broth showed a wrinkled surface growth with the organism climbing up the sides of the flask. At room temperature the growth on agar was not as well developed after five days as it was on the third day at 37.5°C. The broth cultures showed only a sediment on the sides and bottom of the flasks. On agar the cultures were dark in color. In the liquid medium the growth appeared gray.

On the eighth day, at room temperature, the agar cultures were well developed. On dextrose-glycerin broth growth was irregular and somewhat wrinkled on the surface. At 37.5°C., on Sabouraud's agar the growth was becoming opaque, granular and cream-colored. On dextrose-glycerin agar the growth was rougher and dark cream in color. In dextrose-glycerin broth the growth was more accentuated, more rugose and dark cream in color.

Microscopic examination of the primary culture on Sabouraud's glucose agar showed on the fifth day a variety of morphologic forms (Fig. 6B). The organisms were chiefly strongly acid-fast and alcohol-fast, resisting 95% alcohol for five hours without any change in color. After five hours in 3% acid-alcohol, the color showed some fading. In smears, at five days, only a few of the organisms took the stain lightly. Morphologically, the cells were, on the average, slightly smaller than those seen in tissue or pus. The cells measured 0.2 to 0.5 by 1 to 5 microns, showing long and slender, short and thick, club-shaped forms and coccoid bodies. The bacilli were chiefly short, measuring on the average 0.3 by 2.5 microns. Many bacilli showed beading with deeply stained granules. The coccoid bodies were likewise intensely stained. The bacteria were non-motile. The Gram stain revealed Gram-positive granules but the body remained unstained. Repeated attempts with hanging-drop preparations and slide cultures were made to determine whether the organism could produce branching. At no time was true branching found, although there could be seen the pseudo-branching commonly encountered with mycobacteria.

Subcultures were made on various mediums to ascertain the growth characteristics of the organism. All the subcultures were grown in the incubator at 37.5°C. and were made from a three day old subculture growing on dextrose-glycerin agar.

SABOURAUD'S GLUCOSE AGAR (pH 5.6). After 10 days, growth was good. The colonies were smooth, moist, and shiny, light cream in color. On the twenty-first day the colonies had attained a size of approximately 2.0 cm. in diameter and were darker cream in color. The surface of the growth was vemiculate and the border was somewhat lobulate, smooth and shiny (Fig. 4B). Smears made

on the eighth day showed organisms which morphologically were essentially similar to those observed on the fifth day of the primary culture. There were,

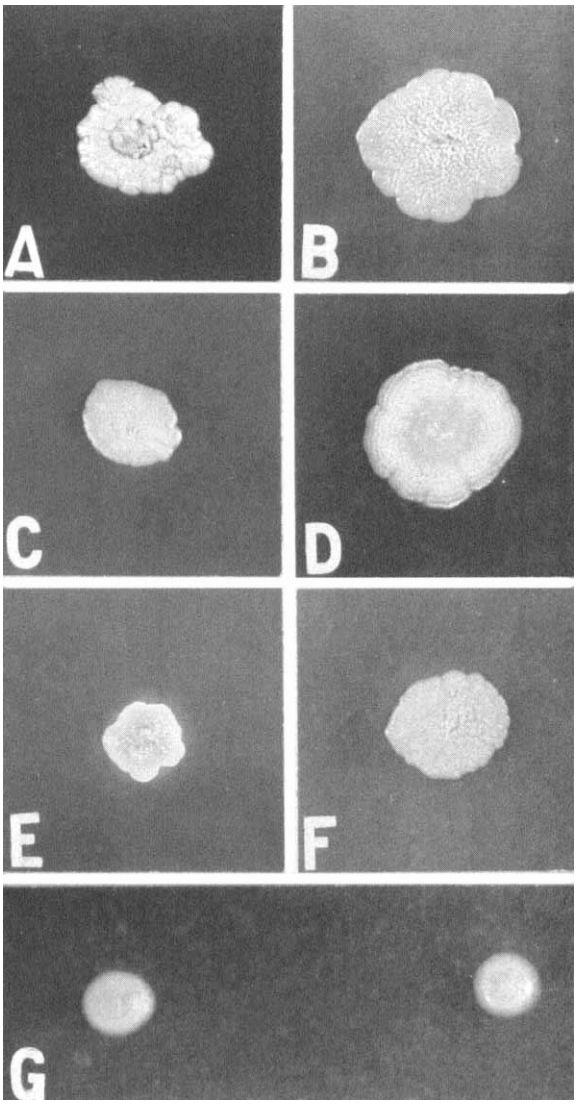

FIG. 4. 21 day old agar subcultures of Mycobacterium abscessus. A. Wort agar; B. Sabouraud's glucose agar; C. Dextrose agar; D. Dextrose agar plus 5% glycerin; E. Nutrient agar; F. Long's synthetic agar plus 5% glycerin; G. Potato-dextrose agar showing umbilicate and mammiliform colonies. × 5.

however, many short beaded forms, some having two granules and appearing somewhat like diplococci. There were also many lightly staining organisms and non-acid-fast forms (Fig. 6A).

In Sabouraud's glucose broth there developed by the eighth day a shiny surface growth which was at first smooth, then somewhat stippled, light cream in color (Fig. 5C). There was some growth also on the bottom of the flask with the broth in between remaining clear. On the tenth day the growth of the organism had extended approximately 1 cm. up the sides of the flask.

NUTRIENT AGAR (pH 6.8). Growth on this medium was slow and poor by the tenth day. The colony was smooth, shiny, and not thick. On the twenty-first day the colony measured approximately 1 cm. in diameter and was grayish-white in color (Fig. 4E). Smears made on the eighth day showed a depauperate type of growth with many non-acid-fast forms (Fig. 6C). The bacilli were variable in size and shape, measuring for the most part 0.2 to 0.3 by 1 to 3 microns. The average cell measured 0.2 by 2 microns. There could be seen poorly stained bacilli with a rather large strongly acid-fast granule.

In broth cultures growth was still poor but better than on agar. On the tenth day there was a thin surface growth, smooth, shiny and gray in color with a thin veil of growth at the bottom of the flask. The broth in between remained clear. The growth on the surface was fragile and sank easily when slightly disturbed.

WORT AGAR (pH 4.8). Growth was good reaching a diameter of approximately 1.5 cm. after 21 days. The culture was lobulate with an irregular periphery, raised, moist and shiny and dark cream to buff in color (Fig. 4A). Smears made on the eighth day showed in the main short, thin bacilli as compared with the plumper bacilli on Sabouraud's glucose agar (Fig. 6F). The organisms measured 0.2 by 1 to 2 microns. There were seen many non-acid-fast bacilli with non-acid-fast granules. The non-acid-fast bacilli appeared to be somewhat longer, measuring 2 to 3 microns in length.

DEXTROSE AGAR (pH 7.3). Growth on this medium was good after a few days, exceeding that of Sabouraud's glucose agar on the tenth day. The rate of growth after the tenth day became slower than that on Sabouraud's agar and the growth reached a diameter of approximately 1.2 cm. after 21 days (Fig. 4C). The colonies were shiny to dull, somewhat rough with an irregular border and dark cream in color. Smears made on the eighth day showed fairly uniform bacilli measuring 0.2 to 0.3 by 1 to 2.5 microns. Some cells were lightly stained and only a few were non-acid-fast. There were very few beaded forms (Fig. 6G).

In dextrose broth (pH 7.2) growth was similar to that on Sabouraud's glucose broth.

DEXTROSE AGAR PLUS 5% GLYCERIN (pH 7.3). This medium gave the best growth for the first four days. The rate of development, however, slowed down so that on the twenty-first day the size of the colony was approximately 2.0 cm. in diameter, similar to that on Sabouraud's agar. The growth at first was moist, then dull becoming rough with an irregular, lobulate periphery. The growth was not heaped up. It soon became dry and flaked off easily with the inoculating needle. On the twenty-first day the growth showed peripheral zonation or concentric ring formation and was dark cream to buff in color (Fig. 4D). Smears made on the eighth day showed numerous beaded cells with strongly acid-fast granules. Many clumps of acid-fast granules could also be seen. The bacilli

varied in size being straight and curved, measuring 0.2 to 0.3 by 1 to 4 microns. The most commonly seen bacillus measured approximately 0.3 by 2.5 microns. Non-acid-fast forms were rare (Fig. 6H).

In broth, growth was fairly rapid, covering the surface with a veil on the fourth day. On the eighth day, the surface growth was thick, dry and extremely wrinkled, dark cream to buff in color (Fig. 5B). The surface growth was fragile with large flakes dropping to the bottom on slight shaking. The broth remained clear.

GLYCEROL AGAR (glycerol 10 gm.; sodium asparaginate, 1.0 gm.; di-potassium phosphate, 1.0 gm.; agar, 15 gm.; tap water, 1,000 cc. pH adjusted to 7.0).

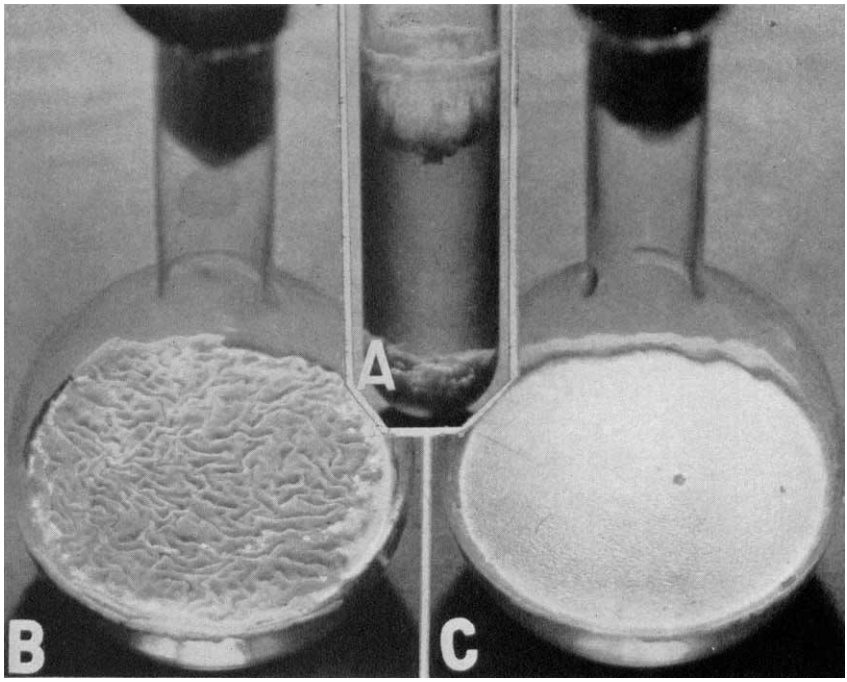

FIG. 5. Broth cultures. A. Dextrose broth plus 5% glycerin, 16 days. Note growth up sides of tube; B. Dextrose broth plus 5% glycerin, 8 days. Shows heavily wrinkled or rugose surface; C. Sabouraud's glucose broth, 8 days. Surface is smooth to somewhat stippled.

Growth was good, similar in gross appearance to that on dextrose-glycerin agar. Smears made on the eighth day showed fairly uniform bacilli with rare beading, measuring 0.3 to 0.4 by 1.5 to 2.5 microns, commonly 0.4 by 2 microns. There were many non-acid-fast forms (Fig. 6D).

POTATO-DEXTROSE AGAR (pH 6.2). Grossly the subcultures on this medium simulated those on Sabouraud's glucose agar although somewhat slower. Single colonies on plates measured approximately 1 to 2 mm. in diameter after 21 days. The colonies appeared either smooth and hemispherical, umbilicate or mammiliform and cream-colored (Fig. 4G). Smears made on the eighth day showed numerous acid-fast granules but no acid-fast bacilli. The body of the bacillus

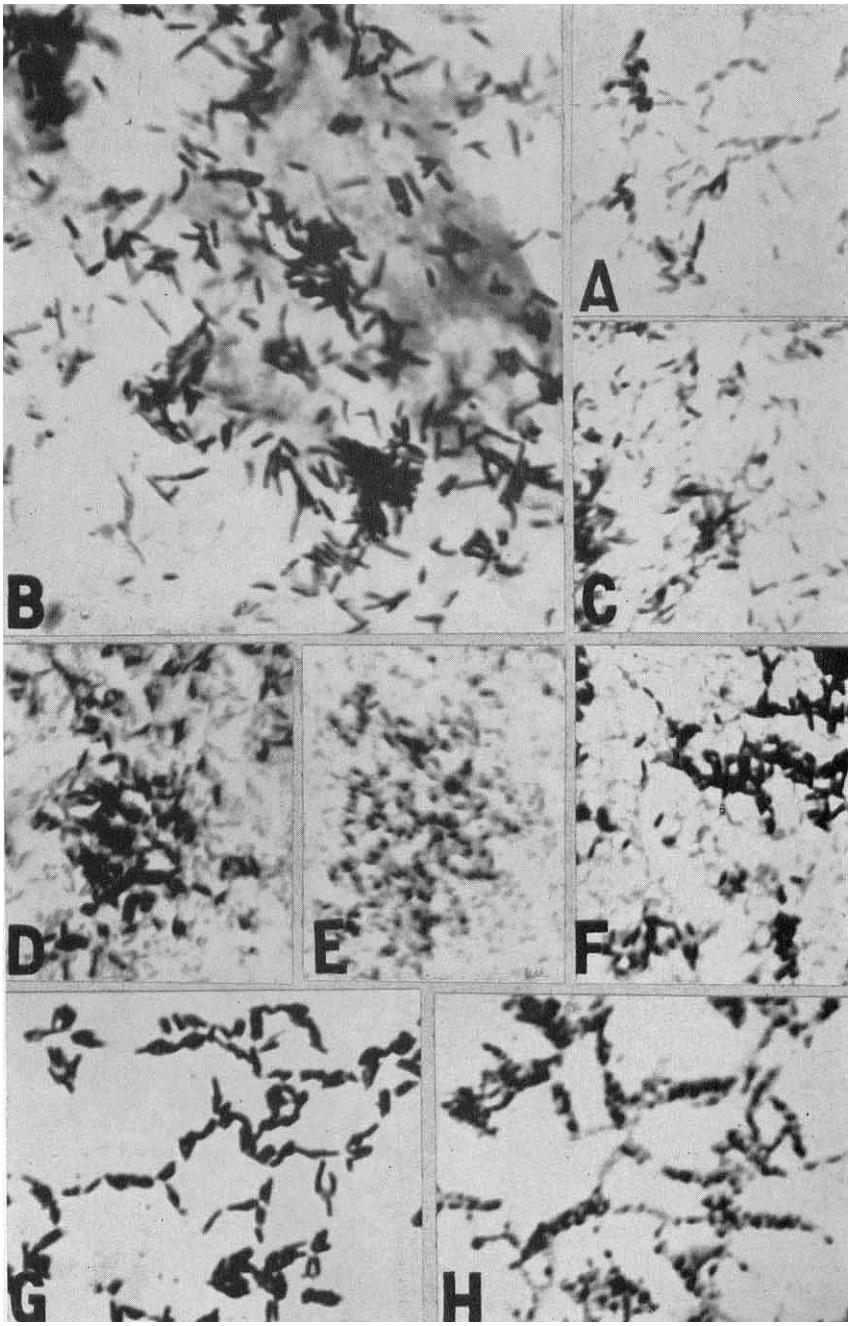

FIG. 6. Culture smears stained by Ziehl-Neelsen method. A. Sabouraud's glucose agar, 8 days. × 2160. Note non-acid-fast forms; B. Primary culture on Sabouraud's glucose agar, 5 days. × 2300. Note fairly uniform morphology; C. Depauperate type of growth on nutrient agar; 8 days. × 2160; D. Glycerol agar, 8 days. × 2160; E. Potato-dextrose agar, 8 days. × 2160. Note granules and absence of stained bacilli; F. Wort agar, 8 days. × 2160; G. Dextrose agar, 8 days. × 2300. Bacilli are uniformly strongly acid-fast; H. Dextrose agar plus 5% glycerin, 8 days. × 2300. Note numerous granules in bacilli.

was non-acid-fast and very lightly stained with the counterstain. There were also some non-acid-fast granules (Fig. 6E).

LONG'S SYNTHETIC MEDIUM PLUS 5% GLYCERIN. Growth on the agar was slow for the first eight days and then increased in rapidity attaining a diameter of approximately 1.5 cm. after 21 days. The growth was somewhat lobulate at the periphery with a pebbled surface and was cream-colored (Fig. 4F).

In broth, no acid was formed. Growth was slow at the start, requiring eight days for the formation of a surface veil as compared with four days on dextrose-glycerin broth. Growth, however, became rapidly abundant with the development of a thick, roughened, deeply verrucous pellicle which was dark cream in color. The pellicle broke easily and dropped to the bottom of the flask as in Sabouraud's glucose broth and dextrose-glycerin broth and further as in these two liquid mediums, growth extended up the sides of the flask for more than 1 cm. Smears made from the growth on the sides of the flask showed as great a variety of morphologic forms as were seen in human tissue. There were long irregular bacilli with beading, as well as short forms. The bacilli appeared, on the average, smaller in diameter, with tapering ends, measuring approximately 0.2 to 0.3 by 1 to 7 microns, with some of the curved bacilli measuring up to 9 microns in length. The average cell measured approximately 0.2 by 4 microns. There were many non-acid-fast forms. The granules in most instances had a larger diameter than the body of the cell.

LONG'S SYNTHETIC MEDIUM WITHOUT GLYCERIN. No growth.

LOEFFLER'S BLOOD SERUM (pH 7.2). Small moist, shiny, cream-colored colonies which became confluent.

GLYCERIN-EGG MEDIUM (PETRIK'S MEDIUM). Growth similar to that on Loeffler's medium.

CZAPEK'S AGAR WITH 3% SUCROSE (pH 4.4). No growth.

TEMPERATURE REQUIREMENTS. Using the method described by Gordon (12) the organism did not survive 60°C. for one hour. It survived 10 and 20 minutes but did not survive 40 minutes at 60°C. when subcultured. The bacillus failed to grow at 47° and also 12°C. It grew well at room temperature, 22° to 25°C. but slowly. Best growth was obtained at 37.5°C., incubator temperature.

CARBOHYDRATE UTILIZATION. The organism grew well in peptone broth containing one per cent carbohydrate but did not ferment glucose, maltose, galactose, levulose, saccharose, d-mannose, mannite, lactose, raffinose, dextrin or inulin.

To determine the utilization of carbohydrates as a sole source of carbon, Merrill's medium as advised by Merrill (13) and advocated by Gordon was used. This is a carbon free medium which adds 0.5% carbon source to the basic medium. After two weeks growth in the incubator there was evidence of utilization of glucose, d-mannose and levulose. There was doubtful growth in maltose, galactose and saccharose. As a check, Czapek's medium was also used. This is also a carbon free medium. In Czapek's basal medium with the addition of one per cent carbohydrate as a source of carbon, the organism showed utilization of the same carbohydrates.

In Long's synthetic medium without glycerin, no growth was obtained. Accordingly, this medium was used incorporating one per cent carbohydrate as the carbon source. There was good growth in glucose, d-mannose and levulose with definite but less growth in maltose, galactose and saccharose.

UTILIZATION OF PARAFFIN. A sterile glass rod dipped in sterile paraffin was placed in a flask of Czapek's medium without carbohydrate to determine whether the organism would utilize paraffin as a source of carbon. The organism developed on the paraffin in the form of a very light yellow growth which covered the rod. Microscopically, the bacilli appeared fairly long and showed numerous granules.

ACTION ON LITMUS MILK. The organism grew fairly well in litmus milk, limiting its growth to the bottom of the tube. The milk was not acidified or curdled. There was an increase in alkalinity as evidenced by the intensity of the bluish coloration.

ACTION ON GELATIN. A gelatin stab showed no liquefaction after several weeks growth. The organism showed a gelatinous type of growth which became somewhat waxy and wrinkled in appearance.

NITRITE REDUCTION. In young nitrate broth cultures, up to 10 days, nitrates were doubtfully reduced to nitrites. In 21 day old cultures, however, there was definite evidence of nitrate reduction.

pH RANGE. The organism grew well in a wide range of hydrogen ion concentration depending upon the medium used. Growth occurred in mediums ranging in pH from 4.8 to 7.3.

ANIMAL INOCULATIONS

CHORIOALLANTOIC MEMBRANE INOCULATIONS. The chorioallantoic membranes of 12 day old fertile eggs were inoculated with 0.1 cc. of a heavy saline suspension of the acid-fast organisms according to a technic described previously (14). Six days after inoculation the membranes were harvested, fixed and sectioned.

Grossly, the membranes showed discrete and confluent grayish plaques or thickenings which were spread over the membranes. Some of these plaques appeared to be raised in the form of small nodules (Fig. 7A).

Microscopically, the involved membranes appeared thickened, showing an inflammatory response with an increased number of capillaries. The ectoderm was variously affected, showing mild involvement in some areas and increased thickening in other areas. In the thickened section there were seen many macrophages with a vacuolated cytoplasm, many containing hematoxylin stained material as seen in hematoxylin and eosin stained sections. When seen in sections stained with the Ziehl-Neelsen method the macrophages were loaded with acid-fast bacilli (Fig. 7D). The ectoderm in some areas showed an ulcerative process with an underlying area of increased reaction.

The infiltrate in the mesoderm, in the areas of greatest activity, was composed chiefly of the vacuolated macrophages, some rather large in size, and numerous basophilic cells which correspond to polymorphonuclear leucocytes. There were also many spindle-shaped cells, probably fibroblasts. At the peripheral zone of some of these areas there were seen ectodermal pearls, evidence of hyperkerat-

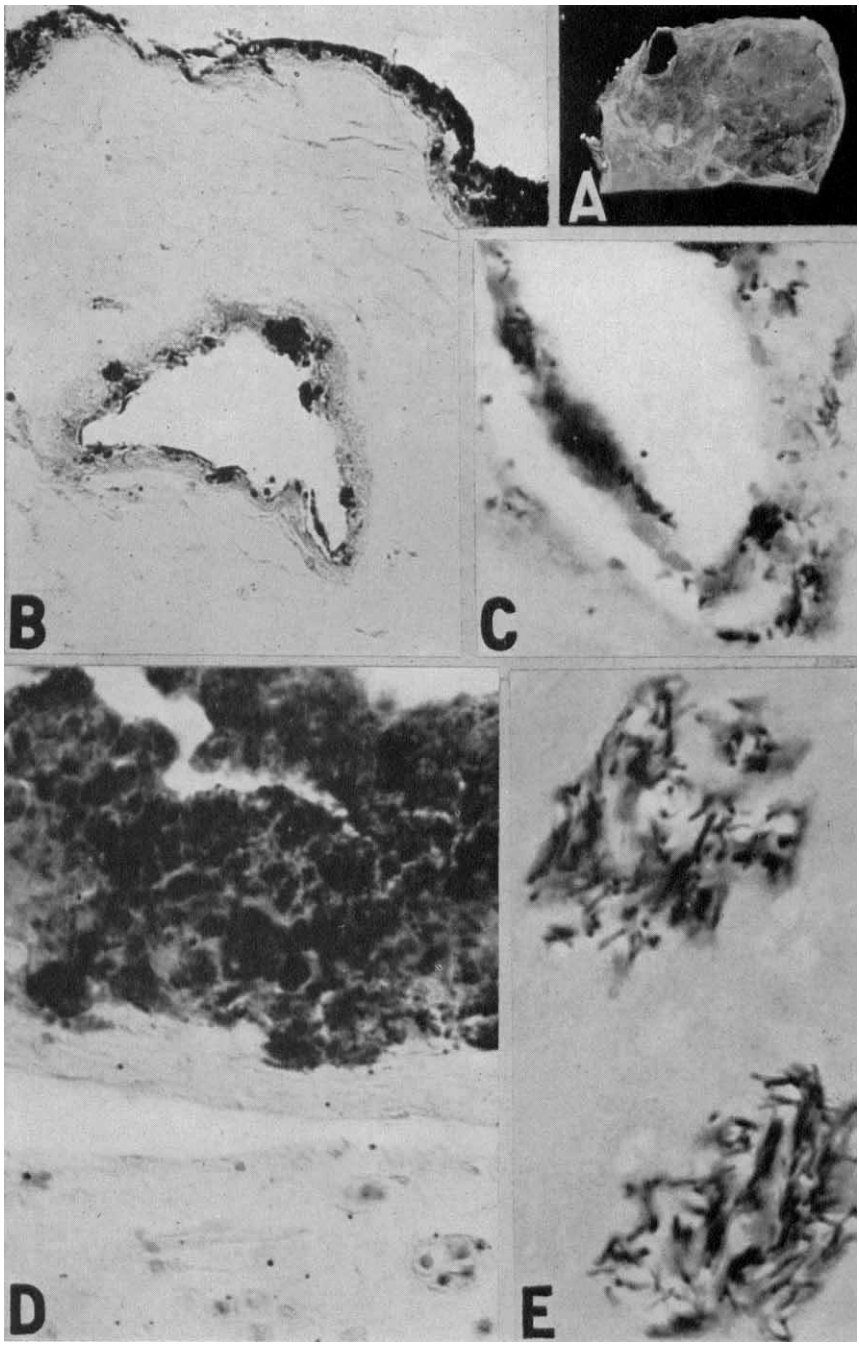

FIG. 7. Chorioallantoic membrane inoculations. A. Whole membrane, showing plaques; B. Section showing blood vessel with infected intima. Z.-N. × 195; C. High power view of intimal involvement showing bacilli. Z.-N. × 1440; D. Involved ectoderm with bacilli in ectodermal macrophages. Z.-N. × 750; E. Clumps of bacilli in amorphous mesoderm. Z.-N. × 1800.

inization. The mesoderm itself appeared somewhat thickened. There were many new capillaries, all packed with red blood cells. A few of the larger blood vessels, especially those close to the ectoderm showed a thickened wall with increased reaction surrounding the vessel. The infiltrate around these vessels was similar to that seen underlying the strong ectodermal reaction, namely vacuolated macrophages, basophilic cells and the spindle-shaped cells. Within the vessel the blood cells were of various types as compared with the almost uniform red blood cells seen in the capillaries.

In similar sections stained for acid-fast bacilli it became evident that the macrophages had migrated deep into the mesoderm from the ectoderm since many of these contained numerous acid-fast bacilli. Of interest also was the finding of one of the large blood vessels with numerous acid-fast bacilli growing in the lining (Fig. 7B, C.).

The ectoderm appeared very little effected except in those areas where the membrane was thin and where the ectoderm was heavily involved. In these areas the ectoderm was thickened with an inflammatory reaction simulating that seen in the ectoderm and underlying mesoderm.

In some sections the chorioallantoic membrane appeared amorphous in character, stained pink and showed very few structural characteristics. The membrane was both infiltrated and surmounted by a massive growth of acid-fast bacilli, appearing in large masses or encased in a clear space surrounded by the amorphous-like substance simulating that seen in the human lesion (Fig. 7E). Interspersed among the bacilli were many cellular elements of the membrane.

LABORATORY ANIMAL INOCULATIONS. Rabbits, guinea pigs, hamsters and mice were selected for inoculation. Thirteen hamsters and six mice received 0.25 cc. intraperitoneally of a suspension of bacilli made by suspending a seven day growth of an agar slant in 10 cc. saline. Three rabbits and 16 guinea pigs received 0.5 cc. intraperitoneally of the same suspension of acid-fast bacilli. Three rabbits and six guinea pigs received 0.25 cc. of the suspension of bacilli intracutaneously in the shaved abdominal wall.

INTRACUTANEOUS INJECTION. Seven days after the intracutaneous injection, the sites of injection of the guinea pigs were warm. The sites had an area of induration varying from 0.3 to 1.0 cm. in diameter with central scabbing. The largest lesion was a central depressed ulcer, 0.4 cm. in diameter in a disc of induration 1 cm. in diameter. The rabbits showed nodules at the injection site measuring 1 to 2 cm. in diameter and approximately 1 cm. in height (Fig. 3B). One nodule showed scabbing. The scab was loosely adherent and it fell off spontaneously allowing thick creamy pus to exude. Smears made and stained for acid-fast bacilli showed many acid-fast rods either freely distributed or in clumps associated with polymorphonuclear leucocytes and some clearly engulfed by macrophages (Fig. 3C). On the ninth day, one rabbit nodule was excised for sectioning. This was split for fixation in both ethyl alcohol and in formaldehyde. The center of the nodule was extremely purulent, more nearly fluid and the roof of the nodule was thin, indicating the possibility of early rupture. On the twelfth day after intracutaneous injection, the guinea pig skin lesions appeared smaller

and showed central scabs. No nodes were palpable. The rabbits also showed smaller nodules including that nodule which had previously ulcerated and still showed a scab.

According to Kite, Patnode and Read, Jr. (15) the criteria for a positive test for virulence for acid-fast bacilli are that a nodule develops which becomes an ulcer in one to two weeks and that there is involvement of the regional lymph nodes. The procedure in their test is to inject 0.01 mg. of bacilli in 0.1 ml. into the skin of the shaved abdominal wall of a guinea pig adjacent to the axillary or inguinal region. A persistent nodule unaccompanied by nodes is indicative of a saprophytic organism.

In our test, 0.25 cc. of a saline suspension from a seven day old agar slant of bacilli was used for the injection. This undoubtedly is a greater dose than that called for in the test. The results, however, would indicate that we are dealing with an organism of low virulence since no regional nodes were palpable even though ulcers did form on the seventh day in several of the animals (both rabbits and guinea pigs).

INTRAPERITONEAL INJECTIONS. *Mice:* An examination of two mice, 12 days after injection, showed no gross lesions with the exception that the spleen of the male was somewhat larger than that of the female. Two other mice, both males, were examined 23 days after injection. Again only the spleen seemed to be larger and there were no gross lesions with the exception of a yellow zone beneath the liver capsule which measured 0.4 cm. in diameter. Two other mice were allowed to live for several weeks longer and when examined showed no gross evidence of any lesions.

Microscopically, the organs of the autopsied mice gave very little information. Wherever there was some fat, near the site of injection, there one could see slight chronic inflammation. This was especially true in the fat associated with the kidney of one mouse and the ovary of another. The livers showed small foci of round cells around many veins and these areas were widely disseminated. In one large vein there was a focus of mononuclears and epithelioid cells.

Hamsters: Thirteen hamsters were used. Eleven were injected intraperitoneally and two served as controls. The hamsters were sacrificed, examined and studied at intervals. Forty hours after injection all organs appeared essentially normal. A piece of liver gave a positive culture for bacilli. On the third day only the omentum appeared affected, showing some thickening. A piece of liver again gave a positive culture although no organisms could be demonstrated in stained sections. On the fourth day the omentum was definitely thickened and inflamed. A positive culture was obtained from the liver. On the fifth day the omentum was thickened and inflamed and there were no apparent lesions in the other organs. Seven days after injection the omentum was still involved and in addition there was a single yellowish-white plaque in the right epididymis and suggestions of a similar lesion in the left epididymis. On the eighth day the omentum was still thickened but no longer reddened. There was a zone of adhesions between the lateral body wall, seminal vesicles and layers of the small intestine. On the ninth day there was seen a small nodule in the peritoneum at the site of injection. There

was also a small yellow nodule, 0.2 cm. in diameter, between the testis and the epididymis on the right side, the injection side. The omentum was slightly reddened. On the tenth day the omentum was questionably thickened and on the twelfth day there were no noticeable gross lesions. Nineteen days after the injection the omentum was adherent to the body wall at the probable site of injection. On the twenty-third day there was a zone of adhesions, in the right lower quadrant, between the small intestine and the abdominal wall. The liver was slightly mottled. There were other adhesions between the loops of the intestine. The lungs were diffusely mottled with red, some of these areas formed confluent blotches. There were a few retroperitoneal nodes which were enlarged to 0.5 cm. There was an opaque yellow spot, 0.1 cm. in diameter, in the papilla of the right kidney. The liver on cut section showed cuffs of paler tissue in the periportal spaces, some measuring 0.1 cm. across.

Hamster tissue, five, 12 and 23 days after intraperitoneal injection was studied microscopically. On the fifth day the omentum was the chief site of involvement. Microscopically there was observed a focal or zonal inflammation in the omental or mesenteric fat. Macrophages were predominant and many of these were seen in epithelioid form. There was a widespread, diffuse infiltration with polymorphonuclear leucocytes with a central zone of early necrosis. With the Ziehl-Neelsen stain organisms could be seen in large numbers in very small foci.

Twelve days after intraperitoneal injection there were no observable gross lesions. Microscopically, however, the organs examined showed noticeable lesions. Abscesses were noted in the pyramids of the kidneys. The spleen showed numerous large cells with inflammation in the surrounding tissue. There were no noticeable lesions in the lungs, prostate, adrenals, heart and testes. In the liver there was a heavy subacute, periportal inflammation. In other areas there were small inflammatory tubercle-like, focal nodules. In the retroperitoneal tissue there was heavy subacute inflammation with caseation which extended to the pancreas.

The hamster sacrificed 23 days after the injection showed a much better gross response to the acid-fast organism. Microscopically the liver showed many small chronic inflammatory foci especially in the portal spaces. With the Ziehl-Neelsen stain no acid-fast bacilli were seen in these foci. A lymph node was found to be hyperplastic but without any focal or tuberculoid lesion. Skeletal muscle taken near the site of injection showed chronic inflammation with a predominance of epithelioid cells. Peripancreatic fat also showed a chronic inflammatory response made up chiefly of epithelioid cells, occasional giant cells and lymphocytes. A number of polymorphonuclear leukocytes were also seen. Mast cells were well dispersed in the tissue.

Acid-fast bacilli were found only in the fat near the pancreas and these were fairly numerous in number. In addition, in the peripancreatic fat, in a nodule, there was noted a small locule with numerous bacilli, as if it were a small culture of organisms. This perhaps may be analogous to what was seen in human tissue and in the chick membrane.

In reviewing the reaction in the hamsters briefly, it is apparent that the reac-

tion occurs chiefly in the fat, particularly that of the omentum and that surrounding the pancreas.

Rabbits: Three rabbits were injected intraperitoneally with 0.5 cc. of a saline suspension of bacilli from a seven day old culture slant. One was sacrificed 12 days after injection, one on the thirty-fifth day and the other was sacrificed nine months later. On the twelfth day, the rabbit, a female, showed small yellow nodules in the peritoneum, the probable site of injection. There were also several small opaque yellowish spots in the omentum. The other organs appeared normal.

One of the rabbits, a female, that had received an intracutaneous injection with the production of an intracutaneous nodule was sacrificed on the thirty-fifth day. There were no noticeable lesions in any of the organs. Both adrenals seemed small but normal in shape and the right adrenal was more normally yellow than the one on the left.

The second rabbit receiving the intraperitoneal injection, a male, was sacrificed on the thirty-fifth day. The spleen showed small, irregular, blue discolorations beneath the capsule. The cut surface showed irregular, very small, gray zones but these were larger than the follicles. The dorsum of the left lung seemed engorged with blood but the lung floated well in the fixative. The liver showed a flat plaque, gray at the edge but opaque yellow and slightly elevated at the center, immediately below the diaphragm and just beneath the liver capsule. In the peritoneum, at the site of injection, there was a dark reddish nodule with an opaque gray rim, measuring 1 cm. across. In the omentum near the greater curvature of the stomach there were multiple small nodules measuring up to 0.5 cm. The lesions of the liver and peritoneum, on microscopic examination, were considered to be parasitic infestations not related to the acid-fast bacilli. There were no noticeable lesions in the thymus, heart and stomach.

The third rabbit, a female, was sacrificed nine months after the injection. Grossly there were seen yellow nodules up to 1.5 cm. in the capsule of the caudate lobe of the liver and nearby peritoneum. On microscopic examination these proved to be parasitic cysts. There were no other noticeable lesions.

Microscopic examination was made of rabbit tissue 12 days after injection. The lung showed no lesions or acid-fast bacilli. The only important section was that of fat and skeletal muscle, comprising a nodule found at the point of entry of the needle into the peritoneal cavity. In the fat were zonal and focal subacute inflammatory lesions. There were many polymorphonuclear cells in these areas but these cells were far out-numbered by mononuclears. The latter cells consisted of both lymphocytes and macrophages, with many of the macrophages developing into epithelioid cells. Langhans type giant cells could be seen rarely. In several of the more tuberculoid lesions, there were necrotic centers and in and near these, acid-fast organisms were numerous (Fig. 9A). Acid-fast bacilli were also seen in non-necrotic more focal lesions but in lesser numbers. Bacilli were not seen in the diffuse inflammation which was made up chiefly of epithelioid cells.

Guinea pigs: Approximately 40 hours after intraperitoneal injection the first pig was sacrificed. All the organs appeared essentially normal except for ques-

tionable thickening of the mesentery. On the third day the second pig showed a thickened and reddened omentum. There was a loose yellowish exudate in the form of small flecks on the splenic capsule. Smears showed numerous acid-fast bacilli and cultures were positive. On the fourth day the omentum was red and slightly thickened. On the fifth day there was some reddening of the omentum and there were several enlarged mesenteric nodes near the large bowel. Seven days after the injection the omentum was slightly thickened and reddened and showed many very small nodules. The epididymis on one side had two yellow nodules 0.1 and 0.3 cm. across. The other epididymis was normal. On the eighth day the omentum was clearly thickened, reddened and adherent to the tip of the liver and some loops of the small intestine. The liver had two areas of paleness beneath the capsule and two very small yellow round zones, 0.2 cm. in diameter, on the ventral surface. On the ninth day the thickened and reddened omentum showed many very small yellowish-white flecks. The liver showed two sub-surface nodules, 0.2 cm. in diameter and one zone of surface reddening. The capsule of the caudate portion of the liver was involved in an inflammatory process along with the nearby omentum. On the tenth day there was noted a pericardial effusion of about 4 to 5 cc. There was pleural effusion on the right side of 6 to 7 cc. with some fluid present also on the left side of the chest. The upper lobes of the lungs were reddened and collapsed. The omentum was distinctly thickened, reddened and showed many small yellowish-white flecks. The spleen was enlarged and had an exudate on its surface. The liver showed many small subcapsular yellowish nodules, the largest measuring 0.2 cm. in diameter. A retroperitoneal node was definitely enlarged to 0.7 cm. in length. Of two female guinea pigs examined on the twelfth day, one showed no lesions and the other had a zone of adhesion in the left upper abdominal quadrant and much fluid on the right side of the chest with the lungs not appearing affected. There were no other lesions. On the fourteenth day the sacrificed guinea pig showed a slight collapse of a segment of the right lower lobe of the lung. The mesentery was greatly thickened and red. The liver showed some swelling and many areas of subcapsular gray discoloration 2 to 3 mm. in greatest dimension but mostly round. One such area was irregular in shape and approximately 0.1 cm. in length. Nineteen days after the injection the omentum was still very thick but only slightly discolored. The testes of this animal were symmetrical but only about one-half normal size. The liver showed a few subcapsular yellow flecks, the largest approximately 0.1 cm. in largest dimension. On the twenty-third day after the injection the omentum was still thick and irregularly reddened. There was a nodule, 0.3 cm. in diameter, on the parietal peritoneum in the lower left quadrant. There were five or six yellow nodules, 0.1 to 0.2 cm. in diameter, beneath the liver capsule. There were adhesions on the under surface of the liver extending to the left lateral body wall and the spleen. The serosa of the stomach was reddened. There were small nodules on the gall bladder. The mesenteric lymph nodes were enlarged to 0.5 cm. Approximately seven and one-half months after intraperitoneal injection the remaining three guinea pigs were sacrificed. These animals showed no lesions, either grossly or microscopically.

Microscopically, the picture varied with the time following the intraperitoneal injection. In some instances microscopic changes were noted where the organs appeared grossly normal. Although all the guinea pigs were examined microscopically, only a few will be presented here in order to indicate the progression of the lesions. On the third day following the injection the lymph nodes showed mild hyperplasia but no focal lesion. The omentum also showed inflammatory lesions but these were not well formed (as in nodules). The peritoneum likewise showed inflammatory lesions. The same held true for the fourth day following the injection. There was a mild, inflammatory reaction without nodule formation in the liver and omentum. The nodes seemed enlarged and showed reactive changes, but no definite focal inflammatory lesions. Acid-fast bacilli were not demonstrable by stain.

On the seventh day there was evidence microscopically of inflammatory nodules in the omentum (Fig. 8A). The center of the nodule was made up of polymorphonuclear leukocytes showing necrosis with some lymphocytes and eosinophilic cells surrounded by epithelioid cells and macrophages (Fig. 8C). In the surrounding tissue there were many capillaries. The whole comprised an inflammatory nodule in a field of cells made up chiefly of epithelioid cells and macrophages but there were no visible giant cells.

On the tenth day, the lungs showed inflammatory changes due to the fluid in the chest. There were no definitely formed nodules. The spleen likewise showed inflammatory changes but no definite nodule formation. On the surface of the liver, penetrating into the tissue, were several small nodules. The early nodule which projected above the surface of the liver was made up almost entirely of epithelioid cells (Fig. 8B), giving thus the yellowish-white appearance. In the later stage there was seen a heavily staining center made up of broken up polymorphonuclear leukocytes (Fig. 8D). Acid-fast bacilli could be demonstrated within and somewhat surrounding this necrotic zone. The omentum showed similar nodules, some without necrotic centers and others with central necrosis. Giant cells were not noted.

Twelve days after the injection, two guinea pigs showed very little grossly. A block of tissue, including skeletal muscle and fat, from the probable site of injection, was studied microscopically. The fat showed extensive lesions of a tuberculoid type. There were no foci of necrosis such as those noted in the omentum. There were, however, many giant cells of both the foreign body and Langhans type. Only where polymorphonuclear leucocytes were seen mingled with the cells of the inflammatory process, which was basically tuberculoid, were there seen numerous acid-fast bacilli. The organisms were found both in the numerous macrophages or epithelioid cells and in the many giant cells (Fig. 9B, C)

On the fourteenth day there was little change in the microscopic picture. On the nineteenth day the omentum was extremely thickened. This increase in size was found to be due to the extensive reaction which consisted of nodules of the type noted on the tenth day. Here, however, the nodules showed not only the necrotic centers (Fig. 8F) but also a tuberculoid arrangement with numerous giant cells (Fig. 8E, G). This was more suggestive of tuberculosis than had been

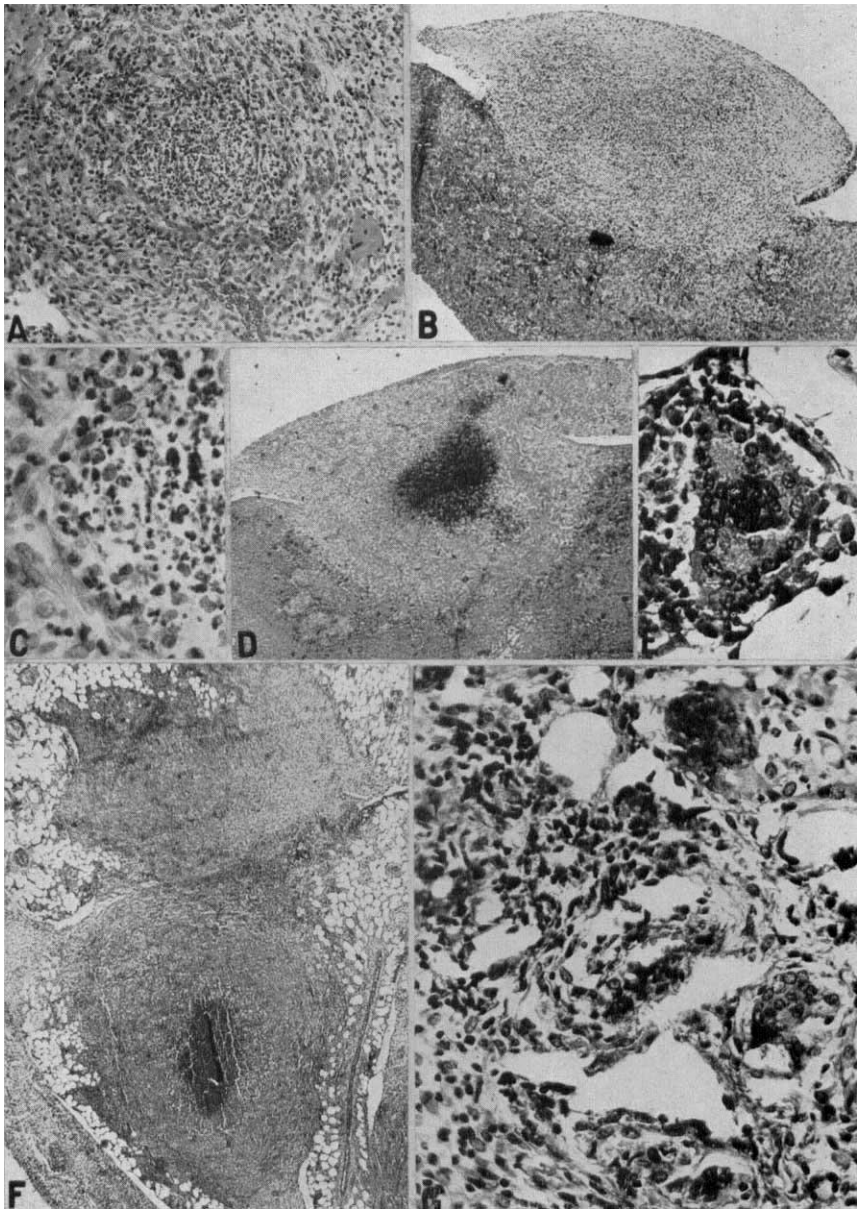

FIG. 8. Guinea pig inoculations. All sections stained with Hematoxylin and Eosin. A. Section of omentum, 7 days, showing an early nodule. × 180; B. Section of liver showing an early nodule made up of epithelioid cells, 10 days. × 78; C. High power view of A. showing cellular infiltrate. × 580; D. Advanced liver nodule, 10 days, showing necrotic center. × 60; E. Giant cell in omentum, 19 days. × 600; F. Nodules in omentum, 19 days. × 60; G. Tuberculoid response in omentum, 19 days. × 495. All magnifications before one-half reduction.

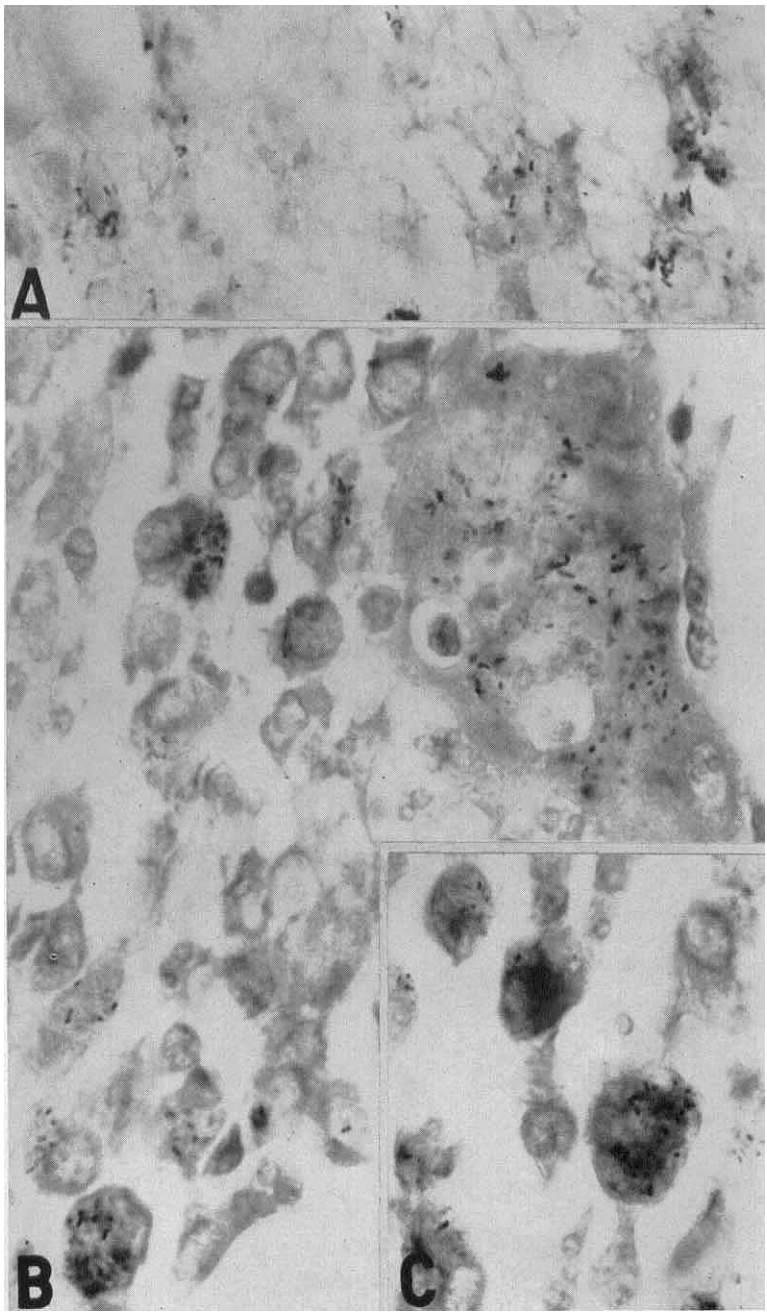

FIG. 9. Organisms in animal tissue. Ziehl-Neelsen stain. A. Area of necrosis near center of nodule, in fat attached to skeletal muscle of rabbit, 12 days. × 1200; B. Bacilli in giant cell and macrophages in guinea pig fat attached to skeletal muscle, 12 days. × 1090; C. Macrophages laden with bacilli in guinea pig fat, 12 days. × 1090.

observed previously. In the liver, both on the fourteenth and nineteenth days, the flecks noted in the gross were nodules identical with those noted on the tenth day. Stains for acid-fast bacilli failed to reveal organisms after several attempts. Tissue observed leveral days later showed a similar type of reaction microscopically and again acid-fast bacilli were not demonstrable.

A recapitulation of the guinea pig tissue response to the intraperitoneal injection of the organism brings out several important points. In the first place, the tissue began to react on approximately the fourth day. On the seventh day the nodules began to form and then chiefly in the fatty tissue, particularly the omentum. In the liver the organism formed nodules which became noticeable on approximately the tenth day. These nodules were made up chiefly of epithelioid cells and developed necrotic centers within which acid-fast bacilli were found to be numerous. Giant cells did not make their appearance until approximately the twelfth day when they were seen in greater number. Acid-fast bacilli were numerous on the twelfth day, being especially present in macrophages and in giant cells. In the omentum, tubercles with necrotic centers continued to be present on the fourteenth, nineteenth and twenty-third days. Lymph nodes were involved but not to the extent noted with virulent strains of tubercle bacilli. The acid-fast bacilli, however, could no longer be demonstrated. It is further obvious from guinea pigs examined at later dates that the tissue reaction did not extend beyond the organs exposed to an intraperitoneal injection. In short, the tissue response reached a level beyond which it did not extend and then fell off, with bacilli no longer demonstrable and eventually the disappearance of the tissue reaction itself. This phenomenon appears to agree with that noted by other observers, using various strains, who believe that the so-called saprophytic mycobacteria are unable to set up by themselves a progressive infection in mammals.

CLASSIFICATION OF ORGANISM

The classification of the numerous acid-fast bacteria is at best a very difficult and unsatisfactory procedure. The definitely established virulent strains producing human, bovine, avian and cold-blooded animal lesions have been rather well studied and classified. There remains, however, an extremely large group of acid-fast organisms isolated from soil and water, some of which may produce disease, which have been inadequately studied and classified. Many of these bacteria, isolated from various sources by different investigators, have either been poorly or inadequately described so that subsequent isolation of a similar organism has resulted in a new or different name. In most instances, no specific name was given the mycobacterium and consequently there are numerous references in the literature to species of mycobacterium known either by the name of the author or the source from which it was isolated. This obviously is more confusing than practical.

In 1932, Thomson (16) made an attempt to classify 26 strains of rapidly growing acid-fast bacteria of diverse origin. She based her grouping on (1) gross cultural features (2) effect of various temperatures of incubation (3) carbohydrate

utilization (4) acid formation from glycerol and (5) cross allergic sensitization of guinea pigs as determined by skin tests. On the basis of these criteria three groups were established.

The organism described in this paper, with some discrepancies would best fit into the second group. In this group, the so-called lepra group, growth was slow on Long's synthetic medium with agar, brilliantly pigmented yellow and orange. Some strains are not pigmented. Growth took place at 40°C., occasionally at 45°C. and not at 50°C. with most of the strains growing at 8° and 25°C. Glycerol, dextrose and mannite served as carbon sources. Some strains grew on Long's medium without glycerin, others did not. Acid was formed from glycerol by only one strain. Skin reactions were obtained on guinea pigs sensitized with one of the members of the group. Cultural and animal tests made this group a less well defined group than the other two. Actually, the differences noted in the organisms placed in this group as compared with the one described here may be sufficiently great to cause confusion. The first and third groups contained too many divergent characteristics to be considered for our organism.

In 1937, Gordon (12) published a classification of acid-fast bacteria which used the following as criteria: gross appearance; growth at 60°C. for one hour; growth in Dorset's medium without glycerin; utilization of carbohydrates; action in litmus milk and nitrate reduction.

On the basis of failure to grow at 47°C., inability to survive 60°C. for one hour and non-utilization of sorbitol and arabinose, our organism would be placed in Group II a_1 of Gordon. There are some differences, however, in carbohydrate metabolism in Merrill's medium with our organism utilizing levulose, glucose, and d-mannose as compared with Gordon's organism utilizing levulose, questionable utilization of trehalose and mannitol and no mention of glucose and d-mannose. In other respects such as lack of utilization of sorbitol and arabinose, alkali production in litmus milk, questionable reduction of nitrates, failure to grow at 47°C. and inability to survive 60°C. for one hour, our organism agrees with the II a_1 grouping of Gordon.

In this group Gordon placed 88 cultures, 66 of which were sufficiently alike in appearance and biochemical reaction to indicate a close relationship. The 66 strains produced a creamy-white growth which did not change color with age. Thirty-five of the 66 were rough in type, producing a crumbly, piled-up growth, the remaining 31 cultures were smooth. Sixty-five cultures were obtained from the soil and one is described as *Mycobacterium ranae* of Küster. The other 22 strains included in this group because of their inability to utilize sorbitol and arabinose vary considerably in appearance and biochemical properties.

In 1938, Gordon and Hagan (17) altered somewhat the classification of Gordon. This revised system has been adopted by Bergey's Manual (18). Accordingly, Group II a_1 includes *Mycobacterium ranae*, *M. thamnopheos*, two strains labelled as *M. leprae* from humans (one the Duval strain and the other the Brinkerhoff strain), 39 unnamed strains isolated from water and 80 strains from the soil. In addition, subgroup II c was created to take care of those strains which were unable to utilize most carbohydrates. In this group they included *Mycobacterium*

chelonei (*M. friedmanni*) from a turtle's lung, *M. schlangen* from a snake, Bayne-Jones' acid-fast organism, Plums' bacillus, Guernsey heifer acid-fast organism, one unnamed strain from the soil and 10 unnamed strains isolated from fish. *M. friedmanni* (*M. chelonei*) deserves further attention since it seems closely related to our species. Culturally the description of this organism has varied according to the investigator. Friedman, Bynoe and Aronson do not completely agree as to color on glycerol agar. According to Merrill, glucose, fructose and arabinose are utilized and according to Gordon, arabinose is not utilized. Bergey's Manual (p. 877), lists the organism as utilizing sorbitol. The chief point of differentiation seems to lie in the optimum temperature which for this organism is 25° to 30°C., while it is 37.5°C. for our species. The discrepancy in carbohydrate utilization of this and other mycobacteria of the group suggests the need for a complete monographic study of these acid-fast organisms. It has been pointed out (Bergey's Manual, p. 883) that the close relationship of the acid-fast organisms producing tuberculosis in cold-blooded animals suggests that they may be one species.

Both *M. ranae* and *M. thamnopheos* seem closely related to our organism. There are differences, however, which are sufficient to distinguish them. *M. ranae* is pathogenic for frogs, lizards and turtles and not pathogenic for rabbits, guinea pigs, rats or mice. *M. thamnopheos* produces generalized tuberculosis in snakes, frogs, lizards and fish, but is not pathogenic for guinea pigs, rabbits or fowls. *M. thamnopheos*, however, may produce lesions of the chorioallantoic membrane of developing chicks (19). In contrast, the organism presented in this paper experimentally produced lesions in rabbits, guinea pigs and hamsters. *M. ranae* cultures on agar are described as having a putrid odor. On glycerol agar, *M. thamnopheos* produces a pale pink to buff growth. Temperatures and carbohydrate requirements of both species differ from each other and also from our species. Because of these dissimilarities and because of other significantly different properties, the organism described in this paper is not synonymous with *M. ranae* or with *M. thamnopheos*.

M. piscium and *M. marinum*, parasites of fish and producers of experimental lesions in fish and cold-blooded animals but not rabbits or guinea pigs, likewise may be ruled out as possible synonyms since both produce colored colonies. *M. piscium* forms thin, flat, smooth, shiny and yellow colonies on glycerol agar and flat, smooth, moist greenish colonies on Dorset's egg medium. In broth this organism forms a thin pellicle with a flocculent sediment. *M. marinum* grows diffusely in broth, forms acid in milk and a deep yellow to orange color on most mediums. These characteristics, in addition to others, differentiate these species from our organism.

In group II a_1 there are also two strains listed as *M. leprae*, those of Duval and of Brinkerhoff. Both strains utilize trehalose, mannitol, fructose and sucrose. The carbohydrate utilization differs from our strain which utilizes glucose, d-mannose, levulose and questionably maltose, galactose and saccharose. It is very questionable whether the two strains actually should be considered as *M. leprae* because of the reported difficulty of obtaining positive cultures. Gordon

and Hagan list 19 organisms described as *M. leprae* (either human or rat) with diversified appearances and cultural characteristics. It is more likely that these organisms may be contaminants of some type since at least five strains resembled soil isolates.

The one distinguishing feature of *M. leprae* in tissue is the fact that the organism grows profusely in the macrophages which engulf it in the form of packets or bundles causing in some cases the formation of globi. Of interest is the observation that in the various lesions produced by atypical acid-fast bacilli, the organisms were usually found in macrophages. It is further of interest that in experimental inoculation whether in guinea pigs or in the chorioallantoic membranes of chicks, the bacilli are invariably taken up by macrophages. In the case of the chorioallantoic membrane, this holds true for human, bovine and avian tubercle bacilli, for *M. marinum* and for *M. leprae muris*. *M. thamnopheos* is difficult to stain in tissue but since there were numerous giant cells found in the infected tissue, it is possible that the bacilli could have been engulfed. In the experimental animal and chick membrane inoculations with the organism reported in this paper, macrophages laden with bacilli were predominant especially on the twelfth day in guinea pigs.

The 39 water and 80 soil isolates were unnamed and consequently will not be considered in the taxonomy of our strain. From the known human pathogens our strain of mycobacterium is easily differentiated on the basis of rapidity and ease of growth as compared with slowness of growth and special medium requirements of the tubercle bacilli. The organism of Johne's disease, *M. paratuberculosis*, requires dead tubercle bacilli or other dead acid-fast bacteria to obtain primary growth. Lepra bacilli have not been grown thus far on known culture mediums.

A comparison with the organisms described in the review of literature should also be made. The strain reported by Cobbett seems to have much in common with our strain. The only difference appears to be the growth of his organism in glycerin broth where it produces a superficial film which remains flat and thin and never wrinkles and does not climb up the sides of the tube. This is in contrast to the growth on glycerin broth of our strain which produces a very wrinkled pellicle and climbed up the sides of the flask.

The Beaven-Bayne-Jones strain likewise shows many characteristics which are in agreement with those of our strain. The chief differences appear to be first that the "Ryan" strain utilizes practically no carbohydrates and consequently was placed in a separate group by Gordon and Hagan. Secondly, on glycerin-egg medium, a thin, glistening, transparent growth developed in 24 hours. This growth became pearl gray, moist and gelatinous, with a yellowish cast. In older cultures, the yellow pigment became tinged with red. This does not happen to our strain.

Unfortunately, Gellerstedt and Englund and Wahlgren were unable to culture organisms from their cases, making comparisons impossible.

On the basis of animal experimentation, the fact that on artificial mediums the organism grew at 33°C. but not at 37°C. and the time required for develop-

ment of growth are sufficient reasons to exclude *M. ulcerans* of McCallum, Tolhurst, Buckle and Sissons as a synonymous bacillus. The same perhaps holds true for the organism isolated by Linell and Norden.

Not enough data were presented by Hellerstrom to make comparisons. The organism described briefly by Pollak and Buhler produced yellow colonies. On the basis of comparison with the various described organisms as presented here and because of the numerous characteristics of the bacillus which differ from the known mycobacteria producing disease in humans, it is felt that we are dealing with a new species of the genus Mycobacterium. Because of the ability of the organism to produce deep abscesses in human tissue it is named *Mycobacterium abscessus*, n.sp.

Mycobacterium abscessus, n. sp. Bacillus in lesions long and narrow, short and thick, straight or curved, ends rounded or pointed, 0.2–0.5 × 1–6 u to 7 u. Coccoid forms 0.5 u in diameter. Bacilli solid, beaded or vacuolated. True branching not formed.

Primary growth on Sabouraud's agar slow at 22°–25°C., faster at 37.5°C. Colonies develop after four days. Colonies small, 0.5 to 2.0 mm. in diameter, smooth, moist and shiny, rounded, mammiliform, light cream in color becoming dark creamy-buff with age. In various mediums cultures smooth to rough, moist and shiny to dull, raised or flat, with or without a lobulate periphery and grayish white to dark creamy-buff in color. Growth best on glycerinated medium. In glycerin broth, pellicle smooth becoming wrinkled or rugose and fragile. The fluid remains clear with a sediment at the bottom of the tube. The cells vary in size and form on different mediums. The cells are strongly acid-alcohol and alcohol fast. Non-acid-fast forms begin to develop on the fifth day.

On Merrill's medium glucose, levulose and d-mannose are utilized. Litmus milk is not curdled or acidified. Gelatin is not liquefied. It decomposes nitrates slightly. It can use paraffin as a sole source of carbon. It grows in a pH range of 4.8 to 7.3. It grows at 22°–25°C. but grows best at 37.5°C. It does not grow at 42°C. and is killed after 40 minutes at 60°C.

Habitat: Observed in synovial lesions and in deep subcutaneous abscesses of the buttocks. It is not fatal to man.

Mycobacterium abscessus, sp. nov. In laesionibus, baculum longum et angustum, vel breve et crassum, aut rectum aut flexum, 0.2–0.5 x 1–6 μ ad 7 μ. Pars terminalis teres vel acuta. Cellulae sphericae diametro 0.5 μ. Bacula solida vel globuli vel vacuolata. Ramos veros non format.

Primus auctus in agar-agar Sabouraudis lentus ad temperaturam 22°–25°C., celerior ad temperaturam 37.5°C. Colonias post 4 dies format. Coloniae parvae, diametro 0.5–2.0 mm., leves, humidae, nitentes, teres, mammiliformes, coloratae dilute cremor ad subalutaceae in coloniea veteres. In mediis diversis, culturae leves vel salebrosae, humidae, nitentes vel obtusae, elevatae vel planes, cum aut sine perimetris lobulatis, albidae ad subalutaceae. Optimus auctus in medio glycerino. In culturis liquidis glycerini, auctus summa liquida levis vel rugosus et fragilis. Fluidum hyalinum, sedimentum ad basem tubuli. In mediis diversis cellulae forma et magnitudine variantes. Cellulae colorantur per fuchsinam acidospirituretificato et spiritu-rectificato haud decolorantur. Cellulae acidospirituretificato decolorabiles in subculturis post 5 dies crescent.

In medio Merrillis glucose, levulose et d-mannose utor potest. Lac coloratum cum litmo

non concretum, acidus nullus. Gelatinum non fluidificans. Decompositionem potassi nitratis parvum provocat. Cera origine sola carbonis utor potest. In pH series 4.8 ad 7.3 crescet. Viget ad temperaturam 22°–25°C., sed viget optime ad temperaturam 37.5°C. Non crescet ad temperaturam 42°C. Decedet de vita post 40 horae sexagesimas partes ad temperaturam 60°C.

Habitat: In laesionibus synovealibus ac in abscessus profundos subcutis clunium observatum est. Morbum hominis non efficit.

SUMMARY AND DISCUSSION

The use of the term saprophyte has always been a source of confusion. When used properly it implies that the substratum upon which the organism is living is already dead and, therefore, incapable of producing any reaction other than a chemical change brought about by the action of the organism. There are undoubtedly a number of such organisms. Many organisms, living in such a state, may under unusual conditions thrive upon a living substratum and produce in it pathologic change. The organism then is referred to as pathogenic. The degree of pathogenesis is dependent upon the relative ability to grow of the organism which is one measure of virulence. When an organism is proved capable of producing disease and thus is a pathogen, it should no longer be referred to as a saprophyte. The literature is replete with reports of saprophytes producing human disease. Obviously this is an incorrect usage of terms.

The case presented in this paper is in point. A girl, 14 years of age, fell and abraded her knee in a farm yard. The knee became infected, obviously a traumatic infection, with an organism living in a saprophytic state, the soil. Although treated by a local physician, the knee lesion did not heal completely and for the next 48 years the patient experienced intermittent pain in the knee joint. At no time during these 48 years was there any evidence that the infectious process had spread beyond the original focus.

At the age of 62 years, the knee became swollen and painful and she had difficulty in locomotion. The surgeons performed a patellectomy and a synovectomy. The tissue was examined histologically and because of the finding of a tuberculoid reaction and acid-fast bacilli, the patient was treated with antibiotics. Following the course of therapy, deep subcutaneous abscesses extending to the surface of the skin were noted in the gluteal region. The supposition was made that these abscesses were a result of the injections. In retrospect, however, we must conclude that the course of development of the gluteal abscesses began when the knee tissue was traumatized. The organism which had been in a semidormant state, since it remained localized, although producing periodic flare-ups in the synovial tissue, had been caused to become disseminated. This spread, almost beyond doubt, was by way of the blood stream and the injections (interestingly, containing streptomycin) served to produce *loci minoris resistentiae.*

Histologically the picture in the synovial tissue was that of a chronic inflammatory reaction with giant cells and epithelioid cells. Acid-fast bacilli were found not in the area of granulation or chronic inflammation but in amorphous pink-staining structures loosely attached to the synovium. In the tibial articular surface there was a soft and mushy area which microscopically showed a central

area of necrosis with amorphous material which was surrounded by a zone containing many giant cells. The finding of acid-fast bacilli with the suggestion of a tuberculoid response led to a diagnosis of tuberculosis and consequently the antibiotic therapy.

In the tissue taken from the gluteal region, the histologic picture was partly similar to that seen in the knee tissue. There were large areas of caseation necrosis deep in the subcutis with areas of chronic inflammation extending upwards to the epidermis. Tuberculoid structures with central caseation necrosis were present as well as small non-caseating tubercles and blood vessels with partial obliterative endarteritis.

In the purulent exudate from the abscess-like areas in the buttocks, bacilli were numerous both in smears where they occurred singly, in clumps both free and in phagocytes and in cultures where they produced numerous colonies. In tissue, although acid-fast granules could be seen in some macrophages, bacilli could not be seen. Reports in the literature agree with this finding. Bacilli could de found in the early stages in the caseous or supurative zones and occasionally in areas of epithelioid cells. In the later stages when the tissue showed a tuberculoid appearance, bacilli could not be demonstrated in the tissue. This was borne out too in the animal experimentation.

The organism in pus obtained from the patient appeared either singly or in clumps, with a variable morphology of long and thin, short and plump and coccoid forms. The bacilli were straight or curved with rounded or tapered ends, showing granules or vacuolations. They were strongly acid-alcohol and alcohol fast. The organism grew easily in a simple medium, Sabouraud's agar, and showed a preference for the incubator temperature, 37.5°C. Colonies developed at room temperature but at a slower rate. On mediums of various ingredients the organism showed a variable type of growth but showed a preference for glycerinated substrates. In broth containing peptone and glycerin, the organism had a tendency to climb up the sides of the flask. When glycerin was present, the pellicle became heavily wrinkled and also quite fragile.

The classification of the organism presented a problem since it did not completely resemble any of the known acid-fast bacilli. It produced no true branching such as one would expect in the actinomycetes. It did grow on Sabouraud's agar, at room temperature, utilized paraffin as its sole source of carbon and grew on a number of mediums which are commonly used for fungi. These criteria have been used by Cuttino and McCabe (20) to help classify their organism with the actinomycetes. In spite of the latter properties of our organism we cannot justifiably place it among the acid-fast actinomycetes because of the absence of true branching. Its position should be among the mycobacteria which, although a controversial group, nevertheless helps to differentiate these unusual organisms. Accordingly it has been classified as a new mycobacterium, *M. abscessus*.

In the experimental animals, in addition to the use of fertile eggs, 22 guinea pigs, 13 hamsters, six rabbits and six mice were inoculated with suspensions of the bacillus. Inoculation of the chorioallantoic membrane elicited the formation of numerous macrophages which easily engulfed the bacilli. Macrophages seem

to play an important role with many experimentally inoculated acid-fast bacilli. Here the macrophages were able to penetrate into the mesoderm and then spread the infectious process to a limited degree.

Intracutaneous inoculation resulted in nodules which ulcerated after seven days. The lymph nodes in the adjacent inguinal region were not affected and consequently the organism was considered to be one of low virulence. In the intraperitoneal inoculations, the mice gave very little information. There was slight diffuse inflammation especially in areas where there was fat. In the hamsters the reaction was more apparent with gross involvement of the omentum chiefly, with changes in the liver, adhesions of the small intestine and enlargement of the retroperitoneal lymph nodes. Microscopically, the reaction occurred chiefly in the fat, especially in the omentum and peripancreatic area. In rabbits too, the chief site of involvement was the fat at the site of the injection, with the formation of zonal and focal subacute inflammatory lesions. In the guinea pig the development of lesions was best. The tissue response became evident on the fourth day with nodule formation on the seventh day particularly in the omentum and on the tenth day in the liver. Acid-fast bacilli were found in great numbers in the necrotic centers of nodules by the tenth day and then in macrophages and giant cells by the twelfth day. The tuberculoid type of reaction made its appearance by the twelfth day becoming exaggerated on the fourteenth day when bacilli were no longer found, and persisting through the twenty-third day. The organism demonstrates well that it is of low virulence since there was no evidence of spread of the lesions and having reached a level of development the lesions began to disappear and the animal remained alive and well.

It is evident from a study of this case and from those presented in the review of literature including the swimming pool lesions with positive cultures, that we are dealing with a new type of mycobacterial disease caused by various acid-fast bacteria of low virulence. This should serve as a warning to pathologists and especially dermal histopathologists not to make a diagnosis of tuberculosis purely on the tissue finding of acid-fast organisms in a 'tuberculoid setting.'

CONCLUSIONS

An unusual acid-fast bacterial infection has been presented with a study of the organism. The lesions produced by the mycobacterium differed because of the duration of the primary involvement (48 years) presumed to be a traumatic infection of the knee, the manner of spread of the organism (hematogenous, following surgery) and the type of lesion produced (subcutaneous, abscess-like lesions with a peripherally tuberculoid structure).

The organism, an acid-fast bacterium, belongs to a fairly large group consisting of isolates from soil and water, many of which are referred to as saprophytes, while others produce lesions in cold-blooded animals, and still others are responsible for low grade infections in humans. Because of its distinctive characteristics of morphology, varied growth on different mediums and biochemical properties which were not completely in accord with other described organisms of the group, we felt justified in naming it a new species, *Mycobacterium abscessus*, of the fam-

ily Mycobacteriaceae. The species name, abscessus, was selected because of the ability of the organism to produce deep, subcutaneous abscesses. These lesions showed caseation necrosis and tuberculoid structures suggestive of tuberculosis.

In animals, including the chorioallantoic membranes of developing chicks, mice, hamsters, guinea pigs and rabbits, the organism produces lesions which, exclusive of those in the chick membranes, are transient. The organism produces a nodular type of lesion with necrotic centers which in turn become tuberculoid and finally disappear, leaving the animal apparently unaffected.

Because it does not produce a progressive disease either in humans or animals, because it tends to disappear from the tissues, apparently ingested by macrophages and finally because the lesions themselves eventually disappear without causing any apparent lasting damage, this organism is considered to be a mycobacterium of low virulence.

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DISCUSSION

DR. HARVEY BLANK, *New York, N. Y.*: Were any skin tests performed with tuberculin in this patient?

I would also like to ask whether this organism resembles the organism that causes Johne's disease of cattle.

DR. JOHN O'BRIEN, *Sydney, Australia*: We have had a few cases of an ulcerating type of skin lesion which we think was caused by an acid-fast organism. This was reported in the Journal of Pathology and Bacteriology within the past three years. The lesions clinically resemble varicose ulcers. The pathologic picture was not suggestive of tuberculosis and the organism was difficult to isolate; however, it could be cultivated at room temperature. Animals were most resistant to inoculation with this organism.

DR. CARL T. NELSON, *New York, N. Y.*: I enjoyed this paper very much. It indicates that there are still some essentially saprophytic acid-fast bacilli which are not described in Bergey's Manual and that some of these microorganisms can induce transient infections in experimental animals and perhaps in man. I do not quite agree that the ability of this bacillus to invade the blood vessels of the chorioallantoic membrane of the chick necessarily signifies that it is hematogenously disseminated in man. Insofar as I can see, this microorganism does not conform to the one Dr. O'Brien mentioned; that is the so-called Bairnsdale bacillus which grows on artificial media only at low temperatures (30 to 33°C).

DR. MORRIS MOORE, *St. Louis, Mo.* (In closing): I would like to say, in answer to Dr. Blank's question, that this patient was tuberculin tested and gave a positive reaction. No further tests were done because we did not think it was tuberculosis. So far as doing tuberculin tests on the animals, we have not done that

yet. We still have work to do and we intend doing that. I do not think it is related to Johne's disease. The organism has characteristics which would place it in a group which has lepra bacilli but I am sure it is not the lepra bacillus. The organism will grow well at room temperature; unfortunately it grows better incubated, but I keep it going constantly at room temperature.

I did not mention previously that the surgeons tried to excise some of the gluteal abscesses and found that the lesion extended into the subcutaneous tissue. They described it as a deep intercommunicating abscess of the gluteal area with extension into the fascial region. They excised everything but the condition recurred in the left gluteal region and spread to the right. I am sure the organism must have spread hematogenously and not by way of the skin because the lesions tend to be present deep in the subcutaneous fat and then work their way upward to the cutis.