TYZZER'S DISEASE SYNDROME IN LABORATORY RABBITS

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Tyzzer's disease was first described 50 years ago as a fatal epizootic disorder in the Japanese waltzing mouse.[1] Subsequently, the disease has been found to occur both enzootically and epizootically in common laboratory mice.[2,3]

The disease occurs most often as a fulminating watery diarrhea in young mice at about the age of weaning.[3,4] It is characterized pathologically by mild to moderate inflammation of the ileum, cecum and colon and by multiple small areas of necrosis in the liver. The diagnosis depends upon the finding of bundles of filamentous Gram negative bacilli (*Bacillus piliformis,* Tyzzer, 1917; *Actinobacillus piliformis,* Wilson and Miles, 1961 [5]) in epithelium of the intestinal mucosa and in parenchymal cells of the liver at the sites of the lesions.[1–4] Although the etiology of Tyzzer's disease has not been clearly established, the results of epidemiologic and transmission studies indicate that the bacillus associated with the lesions is the primary causative agent.[3,6–9] Cultivation of the bacillus on artificial media was described by Kanazawa and Imai in 1959.[10,11] To our knowledge, however, all attempts to cultivate the organism using similar methods in other laboratories have been unsuccessful.

Experimental reproduction of the disorder in the Japanese waltzing mouse has been accomplished by contact exposure and by oral or intravenous administration of infected tissues.[1,12] In other strains of mice, these methods have generally failed, only rarely producing subclinical disease.[8] Tyzzer attributed the resistance of these mice to a genetic factor, which Gowen and Schott [12] later characterized as being dominant. Tyzzer demonstrated in studies of the Japanese waltzing mouse that resistance could also be acquired. The apparent increased susceptibility of common laboratory mice at the time of epizootics has been attributed to the effects of stress.[2] Accordingly, Kaneko and associates [7] found that if common mice were treated with cortisone the disease

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could be consistently reproduced by the oral or intravenous administration of infected tissues. Extending Kaneko's work, Fujiwara and associates produced typical hepatic lesions in cortisone-treated rats and in 10-day-old egg embryos. Attempts to produce lesions in rabbits were unsuccessful.[9]

This report describes the occurrence of a syndrome in rabbits which is remarkably similar to Tyzzer's disease of mice. The disorder, which will be referred to as Tyzzer's disease of the rabbit, occurred in the rabbit colony of the National Institutes of Health. The pathologic features and the results of attempts to isolate the causative agent as well as to reproduce the condition experimentally are described.

Material and Methods

Rabbit Colony. The colony of approximately 1,200 New Zealand white breeders occupies 12 rooms in one wing of the main animal building. Each room contains 90 breeder females and 10 males which are kept, 1 animal per cage, in metal cages with perforated floors. The cages are mounted in 3 tiers on metal racks. Forty adults of the Flemish breed and 25 of the chinchilla breed are caged similarly in an additional room. The removable cage pans are scraped and filled with fresh sawdust twice weekly, and the cages are cleaned in a cage washing machine once every 6 weeks. Water bottles, each fitted with a metal drinking tube, are attached to the outer surface of the cage doors. Food pellets were placed in metal feeders attached to the inner surface of the doors. The non-inbred status of the New Zealand white rabbits is maintained by supplying each room with male and female breeding stock from different rooms. No room contributes to its own breeding stock.

Pathologic Studies. Histologic examinations were performed in 40 rabbits which on gross examination were found to have the typical hepatic lesions of Tyzzer's disease. All of the rabbits were moribund when received and were killed with chloroform. The tissues were fixed in 10 per cent formalin. For special studies some tissues were fixed in Bouin's fluid. All of the visceral organs in 10 rabbits were examined. Only the ileum, cecum, colon, liver and heart in the other 30 were examined. For general survey of the tissues, the sections were stained with hematoxylin and eosin. Giemsa solution (pH 4),[13] the Warthin-Starry Silver method[14] and the periodic acid-Schiff (PAS) reaction[15] were employed to demonstrate bacteria. Harris' hematoxylin,[13] silver impregnation (Warthin-Starry method) and methylene blue (1:5000 in McIlvaine's buffer, pH3) were used as counterstains for the PAS-reaction. Bacterial Gram reactivity was demonstrated with the methods of Scudder, as cited by Lisa,[16] and Brown and Brenn, as cited by Conn, Darrow and Emmel.[17] Diastase digestion was performed with 0.2 per cent diastase of malt U.S.P. (Fisher Scientific Co.) in McIlvaine's buffer, pH 6. Lipids were extracted from formalin fixed sections with chloroform and methanol, equal parts at 60° C for 48 hours. The bacilli found in the rabbit and those in the mouse with Tyzzer's disease were compared morphologically and histochemically. Hepatic tissues from 4 naturally infected mice were supplied in 10 per cent formalin and in Orth's fluid by Dr. Allen W. Cheever (National Cancer Institute, Bethesda, Md.) and Dr. William J. Hadlow (National Institutes of Health, Rocky Mountain Laboratory, Hamilton, Mont.).

Bacteriologic Studies. Approximately 10 per cent suspensions of infected livers in enrichment broths, as well as in physiologic saline and phosphate-buffered distilled water, were used to inoculate media for bacterial growth. The media employed were sheep blood agar, liver extract agar, Eugon, trypticase-soy broth, Omata's

fusiform agar and broth (with and without streptomycin), an agar for lactobacilli,[18] common enteric media (MacConkey, E.M.B., S.S., and desoxycholate), cooked meat medium, and thioglycollate broth with and without agar. Excepting the last two named media, each medium was used aerobically and anerobically. Sterile filtrates of intestinal content from diseased rabbits were diluted with enrichment broths, with and without serum supplement, and were employed as growth media. Chicken eggs embryonated 6 days were inoculated by the yolk sac route.

Disease Transmission. Thirty-eight 3- to 6-week-old New Zealand white rabbits were obtained from a colony in which no evidence of the disease had been observed. They were placed in contact with diseased rabbits or the soiled bedding of diseased rabbits in closed, Horsfall-type cages supplied individually with filtered air. Contact was permitted for 21 days unless death interceded. Since the donor animals (diseased) rarely survived longer than 1 to 2 days and were frequently replaced, exposure to these animals was intermittent for 21 days. The rabbits chosen as disease donors had diarrhea and were littermates of dead rabbits having typical hepatic lesions. The lesions were identified microscopically by examination of frozen sections of liver stained with Giemsa solution. Twenty-one of the exposed rabbits were given cortisone acetate (0.05 mg per gm body weight) subcutaneously on the first day of their exposure.

History of the Disease Outbreaks

The disorder was first recognized during the summer of 1960 as a new diarrheal syndrome which occurred sporadically in entire litters of 3- to 8-week-old rabbits. The profuse, watery to mucoid diarrhea was usually terminated in 12 to 48 hours by death of the animal. Frequently, all of the rabbits in an affected litter died within a week after the first fatality. Only rarely did 1 or 2 rabbits in an affected litter fail to have the diarrheal syndrome or survive it. The dams of such litters occasionally died of the disease after developing a more protracted diarrhea than was ordinarily observed in the offspring. The disease has not yet been found in adult males.

Two major outbreaks occurred: one in the winter of 1960–1961 which involved 9 of the 13 rabbit rooms, and another in the spring and early summer of 1963 which involved all of the rooms. Each outbreak lasted 6 to 8 months. In the interval between the outbreaks the disease smoldered enzootically. Rabbits of all 3 breeds were affected. It is estimated that monthly losses in pre-weanling rabbits were as high as 50 per cent in certain rooms during the most severe months of the outbreaks. In other rooms the losses never exceeded 3 to 6 per cent per month at any time.

In colony surveys, the diagnosis of Tyzzer's disease was based on the finding of typical lesions and bacilli in Giemsa stained frozen sections of the liver. In the study of the pathologic features of the disease, this method of diagnosis was shown to be inaccurate for the purpose of collecting mortality data. In approximately 30 per cent of the diseased animals the hepatic lesions were reparative and contained no bacilli.

In these animals, diagnosis was based on the observation of typical lesions and bacilli in the intestine. The occurrence of ill-defined diarrheal syndromes other than Tyzzer's disease among the rabbits made the collection of clinical incidence data impractical.

Pathologic Observations

Lesions characterizing the disease were found in the cecum, proximal colon, distal ileum, liver and heart. Intestinal lesions were found in each of the 40 animals examined. Most often these were characterized by necrosis of the mucosa and edema of the submucosa and serosa. The necrotic mucosal areas were large and patchy in distribution. They usually involved all of mucosal structures, including portions of the muscularis mucosae (Fig. 1). Edema of the lamina propria and karyorrhexis in scattered glandular epithelial cells were milder changes commonly found in the mucosa between the necrotic portions. Severe submucosal edema was a consistent feature. Many of the thin walled veins and lymphatics emanating from the submucosa and passing through the muscularis externa were choked with cellular debris (Fig. 2). The debris-filled vessels often terminated in, or coursed through, long bands of necrotic smooth muscle in the circular layer of the muscularis externa (Figs. 3 and 6). The bands of necrotic muscle were sometimes visible macroscopically as opaque white lines, up to 2 mm in width; these partially encircled the walls of the distal ileum and proximal colon. The serosal-mesenteric attachments of the intestine were edematous and coated with fibrin. The lymphatic vessels in the mesentery and the sinuses of the mesenteric lymph nodes contained moderate to large amounts of cellular debris.

Leukocytes were few in number in the intestinal lesions of most of the animals. In some, however, the mucosa and submucosa contained moderate numbers of heterophils.

Intracytoplasmic bacilli, which were morphologically identical with those described by Tyzzer, were found in the intestine in each of the 40 animals studied. The bacilli were commonly found in several different layers of the intestine. They were observed in the epithelium in 23 of the animals, in the muscularis mucosae in 39, and in the circular layer of the muscularis externa in 11. Infected epithelial cells usually occurred in portions of the mucosa that were not severely necrotic. The bacilli appeared as bundles of parallel rods or as criss-crossed sticks in the cytoplasm of epithelial cells distributed from the surface of the mucosa to the base of the glands (Fig. 4). In sections colored first by the PAS reaction and then by the Warthin-Starry silver method, silver impregnated bacilli were observed in cells both with and without PAS

demonstrable cytoplasmic mucin. In the muscularis mucosae, the bacilli occurred singly, in pairs, and in small groups in the cytoplasm of smooth muscle cells, with most of them lying parallel to the long axis of the cells (Fig. 5). They were in similar arrangement in the muscularis externa, in partially degenerated smooth muscle cells at the borders of the areas of necrosis (Fig. 6).

The lesions in the liver were punctate areas of parenchymal necrosis which appeared grossly as white spots, usually 2 mm or less in diameter. The number of lesions varied from a few to hundreds in each lobe. Microscopically most of the lesions were located at the periphery of the lobules near the interlobular vessels (Fig. 7). The zone of transition between the necrotic lesion and the healthy appearing parenchyma was thin and consisted of 1 or 2 layers of partially degenerated cells with increased affinity for both basic and acidic dyes. Large numbers of the characteristic bacilli were found in the cytoplasm of these cells and in the more viable appearing cells immediately adjacent to them. They were arranged in the cytoplasm much like those in the mucosal epithelium of the intestine (Fig. 8). Remnants of polymorphonuclear leukocytes occurred in moderate numbers in the necrotic portion of the lesions, and intact heterophils appeared with increased numbers in the hepatic sinusoids. Histiocytes of the liver and spleen were usually heavily laden with necrotic debris. In 12 of the rabbits, all of the lesions observed in the liver were in advanced stages of repair and contained no bacilli. Repair was manifested by fibroplasia within the necrotic portion of the lesion and by the formation of multinucleated giant cells at the border (Fig. 9). Calcified deposits, demonstrable with alizarin red,[19] were commonly observed in the center of these lesions.

In 8 of the rabbits, lesions were found in the myocardium. When observable grossly, these appeared as white streaks 0.5 to 2 mm wide and 4 to 8 mm long extending from the region of the left interventricular groove laterally across the left ventricle. They usually lay near the apex of the heart. Microscopically the myocardial lesions were found in all heart chambers but were more frequently observed in the left ventricle and in the septum. In the ventricles, the lesions appeared as bands of necrotic myocardium which either surrounded myocardial blood vessels (Fig. 10) or were located adjacent to them. The lesions in the atrial myocardium were both focal and diffuse in type and usually involved the entire thickness of the atrial wall. Moderate heterophil response was evident in the necrotic portions of some of the lesions. Characteristic bacilli were noted in partially degenerated (Fig. 11) and normal appearing cells at the sharply delineated borders of the lesions. The bacilli usually lay parallel to the myofibrils. In many heavily infected cells cross-striations were still visible (Fig. 12).

The Bacillus

In formalin fixed tissues stained with acidified Giemsa solution, the bacilli in hepatic cells were basophilic filamentous rods, 0.3 to 0.7 μ wide and 3 to 30 μ long. Most of them, however, measured about 0.4 μ wide and 8 to 12 μ long (Fig. 13). Each bacillus appeared to be enclosed in a colorless cytoplasmic vacuole 1 to 1.5 μ in diameter. The vacuole or halo was evident in frozen-sectioned tissues as well as in tissues dehydrated and sectioned in paraffin. Rarely, long bacilli with subterminal bulbar or fusiform enlargements were found. In some of the thicker bacilli, 0.6 to 0.7 μ wide, colorless spaces occurred at irregular intervals along their length, thus producing the appearance of banding. These bacilli were usually 3 to 8 μ long and had slightly tapered ends. The bacilli with banded appearance and those with subterminal enlargements were demonstrated more clearly when tissues were fixed in Bouin's fluid (Figs. 14 and 15). They were faintly Gram negative and were difficult to demonstrate by either the Gram or the Giemsa method in smooth muscle and myocardium presumably because of their investment with the dense acidophilic muscle cytoplasm.

With the Warthin-Starry silver stain the bacilli were clearly demonstrated in each affected organ as light brown to black rods of different lengths and widths. Silver impregnation increased the diameter of the thinnest bacilli to about 0.6 μ. The cytoplasmic vacuole or halo around the organism was evident only in heavily impregnated tissues. Banding was demonstrated best in lightly impregnated tissues. Subterminal enlargements, which occurred in long slender bacilli, appeared as distinct bulbar or fusiform swellings with no observable internal structure (Fig. 16). The bacilli with subterminal enlargements were relatively few in number. In some of the rabbits, none were found. They usually occurred singly in cells that also contained organisms without enlargements. They sometimes occurred alone in cells that were in advanced stages of degeneration. Many short, thick bacilli had an enlargement at one end appearing as an elongated, ovoid swelling constituting a third to a half of the length of the bacillus (Figs. 16 and 17). The end of the enlargement was often tapered to a dull point. Commonly, the terminal enlargement contained either no demonstrable internal structure or a thin straight line of silver impregnated material which lay in the center of the enlargement parallel to its long axis. Others contained a large, heavily impregnated, bar-shaped body which occupied the entire central area of the enlargement (Figs. 18 and 19). The bacilli with terminal enlargements usually occurred in clusters of 2 to 6, often in cells that contained other bacilli without enlargements (Figs. 19 and 20).

Many of the bacilli were demonstrated well by the PAS reaction. They appeared as chains of irregularly spaced PAS-reactive beads and rod-shaped bodies of varying length and thickness (Figs. 21 and 22). The PAS-reactive bodies of most of the bacilli measured 0.3 μ or less in diameter. Chains of larger bodies, up to 0.7 μ in diameter, were observed in some. Secondary staining of the bacilli with Giemsa solution usually concealed the smaller bodies. The larger bodies remained visible and coincided in position with the colorless spaces of the Giemsa stained banded bacillus described previously. This finding was shown most clearly by comparing photographs of Giemsa stained bacilli with photographs of the same organisms after they were decolorized with ethanol and recolored by the PAS-reaction (Figs. 23A and 23B). Some of the slender filamentous bacilli contained only small beads of PAS-reactive material barely large enough to be observed. These were demonstrated best when hematoxylin was used as a counterstain. Hematoxylin outlined the bacilli faintly and caused no perceptible interference with observations of the PAS-reactive bodies. In each of the rabbits, at least 5 to 10 per cent of the bacilli shown with hematoxylin contained no observable PAS-reactive material. The bacillus with a subterminal enlargement consisted of a long chain of slender, rod-shaped, PAS-reactive bodies with one very wide body at the site of the enlargement (Fig. 24). The wide body sometimes appeared to be comprised of 2 rod-shaped bodies located side-by-side (Fig. 25). The short, thick bacillus with a terminal enlargement contained 2 to 4 large PAS-reactive bodies in its bacillary part. The bodies located nearest to the terminal enlargement usually were the largest. That located immediately adjacent to the enlargement was often cup-shaped and seemed to form a cap over the end of the enlargement.

In contrast with the subterminal enlargement, the terminal one contained either no PAS-reactive material or a small amount which appeared to coat incompletely the inner surface of its walls (Fig. 26). Methylene blue was the most satisfactory counterstain for studies of the PAS-reactivity of the terminal enlargement. It outlined the enlargement by negative coloration and lightly stained its internal structures. Both Giemsa and hematoxylin stains failed to demonstrate terminal enlargements. Although silver demonstrated the enlargement well and was used successfully as a counter-colorant, the impregnation process usually reduced greatly the intensity of PAS staining.

The PAS reaction and the Warthin-Starry silver method were employed to demonstrate the bacillus of Tyzzer's disease in the mouse. The organisms in the mouse contained PAS-reactive bodies which appeared indistinguishable from those found in the rabbit. The PAS-reactive

bodies in both animals resisted malt diastase digestion at 37° C for 60 minutes and were not visibly altered after the sections were soaked in a solution of equal parts of chloroform and methanol at 60° C for 48 hours. With silver impregnation, the bacillus found in the mouse appeared more distinctly banded than the one in the rabbit. Bacilli with terminal enlargements were fewer in number and none with subterminal enlargements were found.

Experimental Disease Transmission

Transmission of the disease was accomplished by the contaminative method as described above. Of 11 rabbits exposed intermittently to the diseased ones for 21 days, 2 died, one on the ninth day and one on the tenth day after the onset of exposure. Diarrhea began in these animals 4 days prior to their death. Both animals had lesions and exhibited bacilli typically like those of Tyzzer's disease. Nine of the rabbits survived the exposure period without evidence of disease. No lesions were found macroscopically when the survivors were killed on the 21st day.

Six additional rabbits were given cortisone (0.05 mg per gm body weight) subcutaneously at the beginning of their exposure to the diseased rabbits. Each of them developed diarrhea and died in 6 to 9 days. The meshed flooring of the cage was then removed and 15 cortisone-treated rabbits and 6 untreated rabbits were placed in the cage in direct contact with the soiled bedding. All of the cortisone-treated rabbits died of the disease within 5 to 11 days following the onset of exposure. Diarrhea usually occurred in these animals less than 24 hours prior to death. The 6 untreated rabbits survived 21 days of exposure without evidence of disease. Of 11 cortisone-treated animals that were not knowingly exposed to diseased animals or to contaminated materials, 1 died 10 days after the treatment. Typical lesions and bacilli were found microscopically.

The disease is now being passed successively in cortisone-treated rabbits by exposure to contaminated bedding. An attempt to transmit the disorder to 20 NIH general purpose mice of weanling age by this method was unsuccessful. In future studies, other strains of mice will be exposed and attempts will be made to produce the lesions in mice and rabbits by oral and parenteral administration of infected tissues.

Attempts to Isolate the Etiologic Agent

Many types of bacteriologic media were inoculated with suspensions of infected liver from the diseased rabbits. Although a wide variety of bacteria were isolated from the livers, none were found to have the

morphologic properties of the bacillus associated with the lesions. The bacteria isolated were coliforms and other natural inhabitants of the intestine which presumably invaded the host at the site of the intestinal lesions. When liver suspensions were injected into embryonated eggs, growth of these agents invariably resulted in death of the eggs in 24 to 48 hours. Attempts to prepare uncontaminated inocula are in progress.

Discussion

The hepatic lesions in the rabbits resembled those in mice affected with Tyzzer's disease. The morphologic and tinctorial properties of the bacillus associated with the lesions were like those reported by Tyzzer and subsequent investigators.[1,8,9] As in Tyzzer's disease in the mouse, the bacillus occurred in the cytoplasm of parenchymal cells bordering the hepatic lesions and in intestinal epithelium in the distal ileum, cecum and proximal colon. Our failure to cultivate the bacillus with a wide variety of artificial media only further likened the organism in the rabbit to that in the mouse. Transmission of the disease was accomplished by the contaminative method as described by Tyzzer in studies of the Japanese waltzing mouse. The results of the transmission attempts were improved maximally when cortisone was administered to the rabbits as prescribed by Kaneko and associates[7] and Takagaki, Naiki, Fujiwara and Tajima[8] in studies of Tyzzer's disease in the relatively resistant common laboratory mouse. The clinical episode of fulminating watery diarrhea in weanling animals constitutes another similarity of the disease syndrome in rabbits to Tyzzer's disease of mice. Thus, on the basis of the evidence at hand, we feel that the syndrome should at least tentatively be called Tyzzer's disease of rabbits.

The lesions were more widely distributed in the rabbit than in the mouse, occurring in myocardium and in intestinal smooth muscle. These lesions, however, had the same basic characteristics as the hepatic lesions of both animals; i.e., they were sharply localized areas of necrosis near vessels and contained filamentous intracellular bacilli in their bordering cells. In both the rabbit and the mouse, it is likely that the bacillus is transported from the intestine to the liver by way of the portal circulation. In the rabbit, it appears that the intestinal lymphatics also are involved, thus providing a route of infection to the heart which bypasses the liver. Blockage and injury of the thin-walled intestinal lymph vessels by infected cellular debris may be important in the development of the smooth muscle infection and the edema of the intestine. The accumulation of cellular debris in the sinuses of the mesenteric lymph nodes also may contribute to the development of intestinal edema.

Tyzzer's findings indicate that infection of smooth muscle and myocardial cells may have occurred in the waltzing mice. He reported the occasional finding of bacilli in the walls of blood vessels in the intestinal mucosa and in the lumen of intestinal vessels which he believed to be lymphatics. Also, he reported the occurrence of a myocardial lesion in a mouse given infected liver suspension intravenously; its microscopic appearance was not described. Presumably, such lesions of muscle have not been observed in association with Tyzzer's disease in common laboratory mice. It should be emphasized that the myocardial lesions of the rabbit are not easily recognized grossly and that lesions in the muscularis mucosae of the intestine are inconspicuous even when observed microscopically. Although the lesions in the muscularis mucosae are localized, they are poorly discernible because the muscularis mucosae is very thin and because its cells remain viable in appearance in spite of heavy infection by the bacillus. Of the lesions in muscle, only those in the muscularis externa were striking in appearance both grossly and microscopically. The identification of lesions in muscle was complicated by the fact that the bacilli in muscle cells were demonstrated poorly or not at all by Giemsa or Gram's solutions. Evidently, the basic proteins of muscle cytoplasm impede the diffusion and absorption of basic dyes sufficiently to prevent adequate coloration of the bacillus. The bacilli were demonstrable with the PAS reaction, but were easily overlooked in the PAS-reactive cytoplasm of injured muscle cells. They were consistently shown well only with the Warthin-Starry silver method, which apparently has not been used in studies of Tyzzer's disease in the mouse. With this method, the bacilli were found in the muscularis mucosae of the intestine in 39 of the 40 rabbits studied.

As in Tyzzer's disease of the mouse, the consistent finding of the characteristic intracytoplasmic bacillus in association with the lesions suggests that the bacillus is the causative agent of the disease. The suspected etiologic role will be difficult to prove, however, until serologic or disease transmission studies are performed with the isolated bacillus. The infective materials used in our transmission studies to date may have contained a variety of agents other than the bacillus in question. However improbable it may seem, the possibility exists that the bacillus may be simply an opportunist which has become a convenient diagnostic criterion.

Also difficult to assess is the problem of whether the bacillus observed in the rabbit is the same one found in the mouse. Neither bacillus has been adequately characterized microbiologically. The infectivity of the rabbit's bacillus for the mouse, and *vice versa,* has not been critically examined. There may be several similar or closely related bacilli, each

capable of causing or being associated with the lesions of Tyzzer's disease. It should be noted that the results of our diastase digestion studies on the bacillus from both the rabbit and the mouse in this country contrast with those reported from Japan by Fujiwara and coworkers.[9] Fujiwara observed that the PAS-reactive material in the bacillus from the mouse was diastase digestible. The bacilli shown in his photomicrographs appear to have PAS-reactive bodies like those we have observed.

Bacilli with spore-like enlargements similar to those described by Tyzzer are commonly observed in the rabbits. The subterminal and terminal enlargements are morphologically and histochemically different. The subterminal ones occur in long slender bacilli, are commonly demonstrable with Giemsa stain, and contain a large centrally located mass of PAS-reactive material. In contrast, the terminal ones occur in short thick bacilli, are not demonstrable with Giemsa stain, and contain either no PAS-reactive material or a small amount which appears to be adherent to the walls. Whether either of the enlargements function as spores is not known. Tyzzer's studies of the bacillus in the mouse suggest that one or both of the enlargements may function to preserve the bacillus in dormant state. He observed that contaminated bedding remained infective after a year of storage at room temperature. Fujiwara, Fukuda, Takagaki and Tajima[20] examined the enlargements electron-microscopically and described them as "spore-like" bodies comparable in position to the Clostridial spore. As pointed out by Tyzzer, the results of his studies on the thermostability of the bacillus are inconclusive. Although the infectivity of diseased tissues was apparently lost as a result of heating to 80° C for 1 hour, resistant forms may have been present in the tissues in numbers too low to cause fatal disease when administered to animals. Also, the test employed by Tyzzer may have been too severe. McClung and Lindberg[21] stated that "Cultures surviving 20 minutes heating at 80° C may be presumed to be sporeformers." Furthermore, it is questionable now whether heat resistance may be used as a limiting criterion for defining bacterial spores. In a recent study of 83 strains of *Clostridium perfringens,* Hall, Angelotti, Lewis and Foter,[22] found that although each strain produced microscopically observable spores, the spores of only 30 strains were heat resistant (surviving 100° C for 30 minutes). The infected tissues which Tyzzer subjected to heat were ground and suspended in saline. Subsequently, Kaneko and associates[7] showed that fluid suspensions of the diseased tissues were greatly reduced in infectivity after storage at room temperature for only 2 to 5 hours. Since the bacillus of both the mouse and the rabbit evidently survive for extended periods of time

at room temperature in contaminated bedding, it appears that many factors aside from heat such as pH, oxygen tension and moisture of the suspending medium may greatly influence the stability of the bacillus.

The PAS-reactive material in the bacillus was not removed by chloroform-methanol extraction or by exposure to diastase. The material possessed no detectable affinity for basic dyes in solutions of pH 3 to 4. These findings indicate that the PAS-reactive material is composed of simple sugars such as those found in neutral mucins.[23,24] One of the cells invaded by the bacillus, the intestinal epithelial cell, has the capacity to produce both neutral and acidic mucins.[25] The 3 remaining cells invaded by the bacillus (smooth muscle, hepatic and myocardial cells) are noted for their capacity to synthesize and store glycogen.[26] It is suggested, therefore, that the cellular selectivity exhibited by the bacillus may be related in some way to the capacity of the invaded cells to metabolize sugars.

Since Tyzzer's disease has not been reported heretofore as a naturally occurring disease in the rabbit and is not known to have occurred in the 30-year-old rabbit colony at the National Institutes of Health prior to 1960, its sudden appearance suggests that the causative agent was newly introduced. Its occurrence in epizootic form indicates that the population of pre-weanling animals was initially highly susceptible. The rare occurrence of the disease in older animals during such outbreaks, especially during the first outbreak, implies the development of natural resistance with age. The regression of epizootics suggests that the susceptibility of the animals was modified, probably through exposure to the agent. We know of no unusual stress that may have favored the development of epizootics. How the agent was introduced into the colony is not known, nor is it known whether the disease was spread by means other than contact with diseased animals or contaminated bedding. If carrier states existed, in the more resistant adult animals, the moving of breeder animals to different rooms may have introduced the agent into many rooms prior to the epizootic episode.

Summary

Two epizootic outbreaks of a previously undescribed transmissible disease of rabbits occurred in the rabbit production colony at the National Institutes of Health. The disease occurred as a fulminating watery diarrhea in litters of 3- to 8-week-old rabbits. Pathologically the disorder was characterized by (1) edema and necrosis of the mucosa and submucosa of the distal ileum, cecum and proximal colon, (2) multiple small areas of necrosis at the periphery of hepatic lobules, and (3) in many of the rabbits, localized areas of necrosis in the muscularis ex-

terna of the intestine and in the myocardium. Filamentous, Gram negative, intracellular bacilli which often possessed a terminal or subterminal enlargement were observed in the affected organs in each of the rabbits. They were found in the cytoplasm of hepatic, smooth muscle, myocardial and intestinal epithelial cells bordering the lesions in the respective tissues. Most of the bacilli contained a chain of irregularly spaced PAS-reactive bodies which resisted diastase digestion and chloroform-methanol extraction. The bacilli and hepatic lesions were believed to be indistinguishable from those of Tyzzer's disease in mice. The lesions in the intestinal smooth muscle and myocardium were of the same basic type as those observed in the liver.

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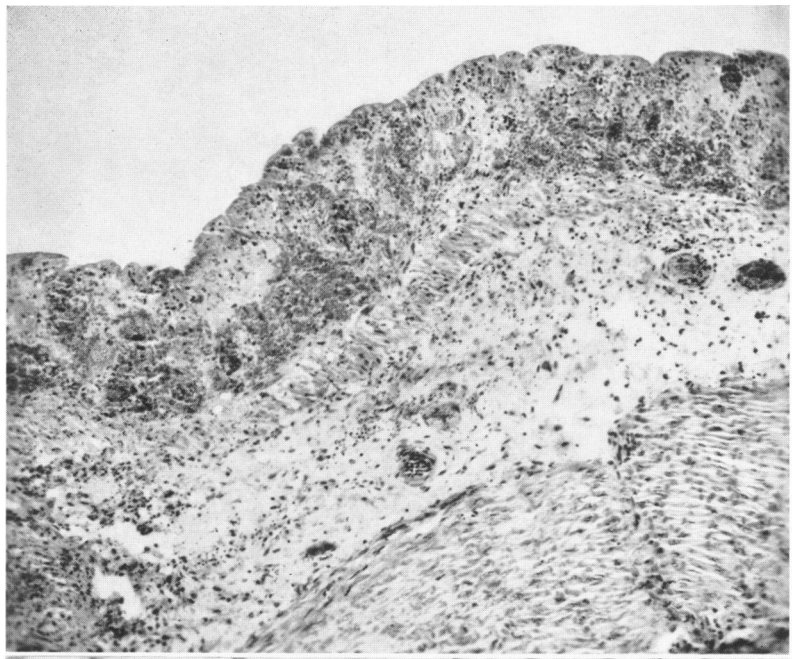
LEGENDS FOR FIGURES

FIG. 1. Proximal colon with necrotic mucosa and edematous submucosa. Hematoxylin and eosin stain. × 115.

FIG. 2. Debris laden lymph vessel in the circular layer of the muscularis externa of the colon. Hematoxylin and eosin stain. × 385.



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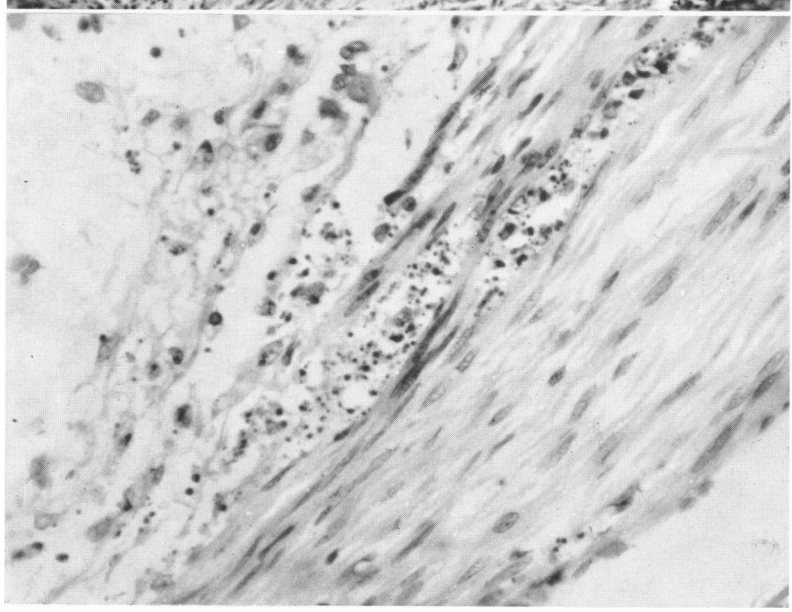

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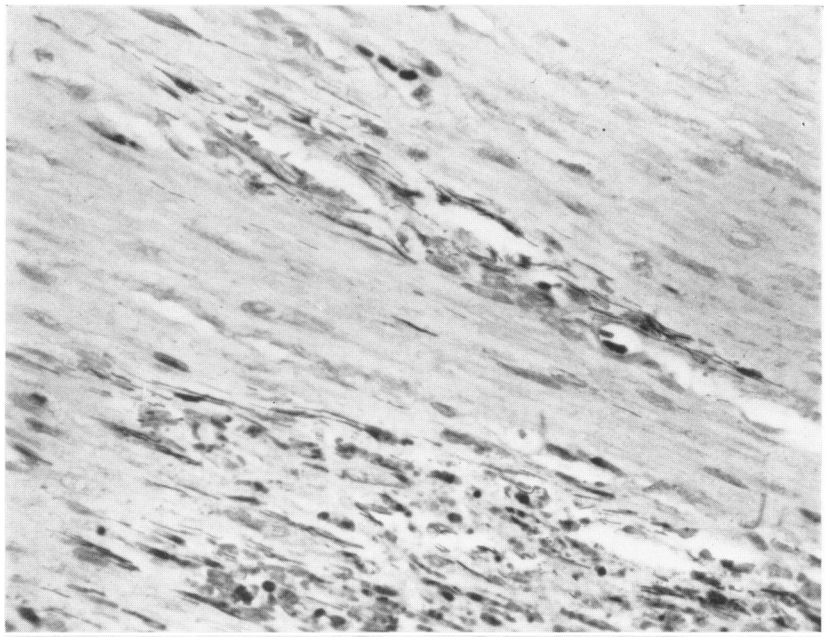
FIG. 3. One end of a sharply delineated zone of necrosis in the circular layer of the muscularis externa (proximal colon). Bacilli are evident in the bordering cells and in the cells adjacent to the lymphatic above the lesion. Warthin-Starry silver stain. × 450.

FIG. 4. Bacilli in the cytoplasm of glandular epithelium of the cecum. Warthin-Starry silver stain. × 880.



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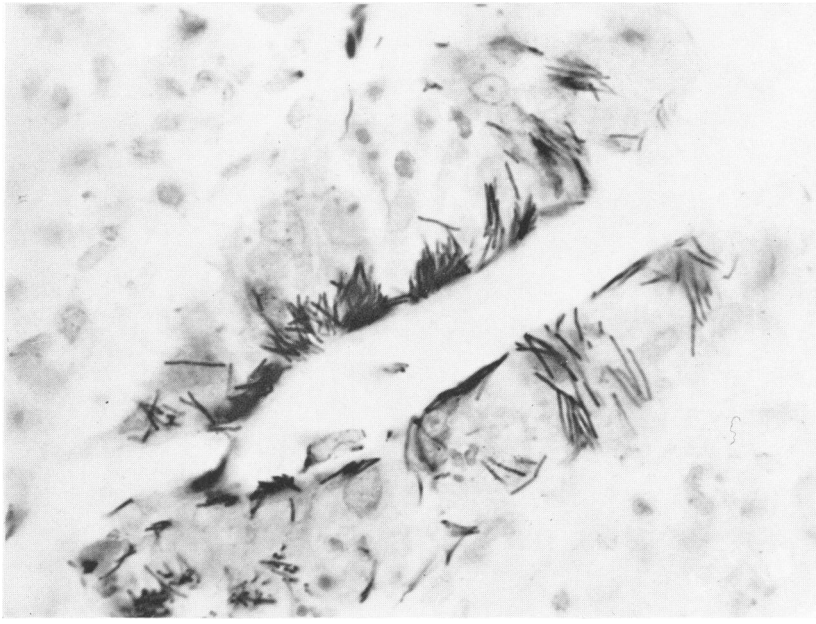

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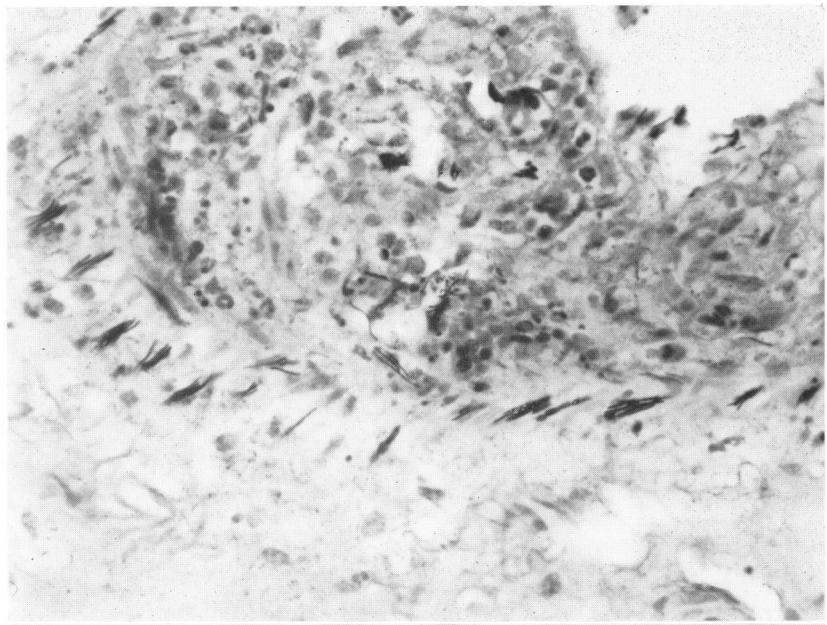

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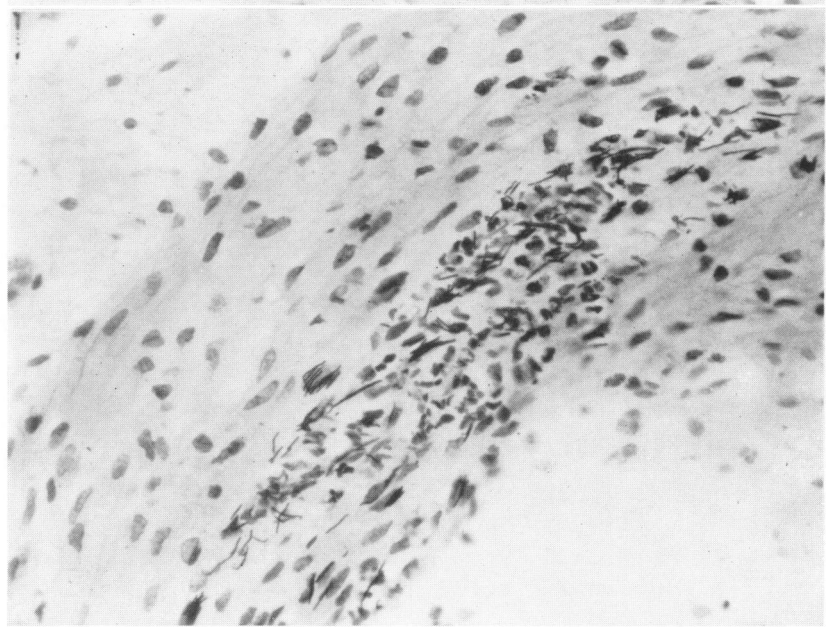

FIG. 5. Necrotic mucosa in the distal ileum. Numerous cells of the muscularis mucosae contain typical filamentous bacilli. Warthin-Starry silver stain. × 635.

FIG. 6. Circular layer of the muscularis externa in the ileum. The cells bordering a small zone of necrosis contain many intracytoplasmic bacilli. Warthin-Starry silver stain. × 450.

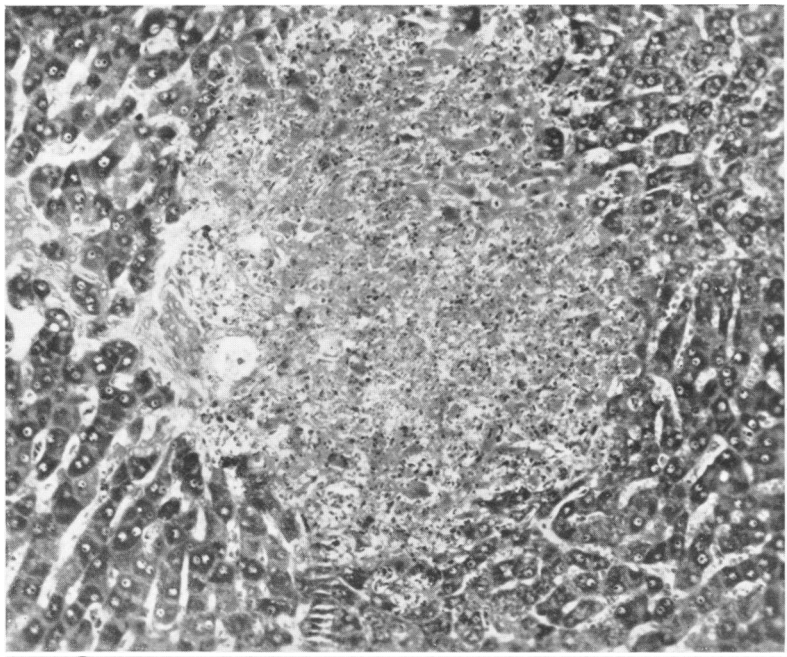

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FIG. 7. Localized area of necrosis in the liver adjacent to the interlobular vessels and bile ducts. Giemsa stain. × 195.

FIG. 8. Bacilli in the cytoplasm of hepatic cells which border an area of necrosis. Warthin-Starry silver stain. × 1,450.

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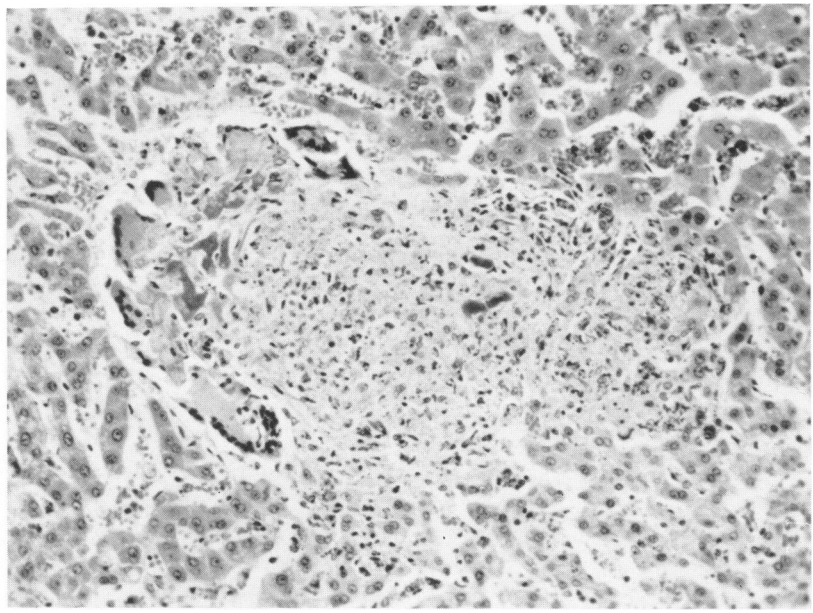

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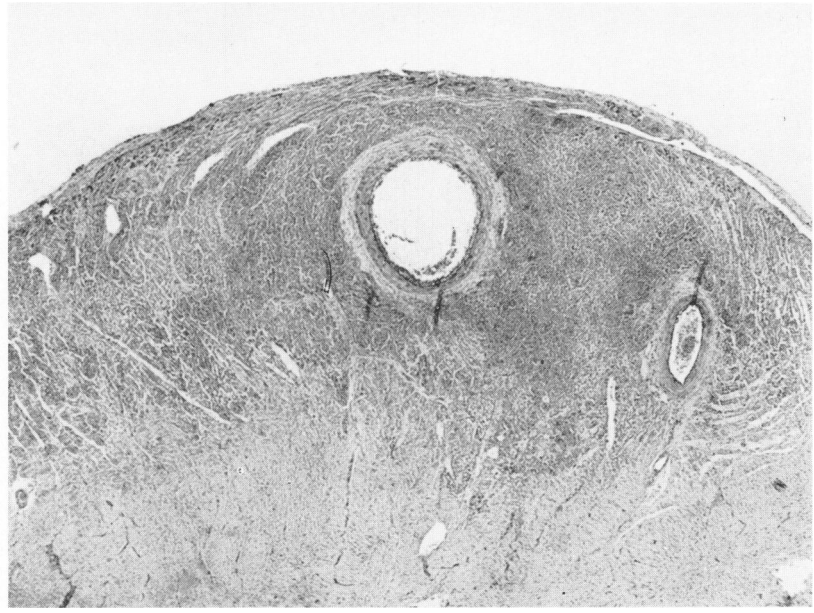

FIG. 9. Hepatic lesion in advanced stage of repair with evident fibrosis and giant cell formation. Hematoxylin and eosin stain. × 195.

FIG. 10. Heart, left ventricle. A large necrotic area surrounds several myocardial vessels. Hematoxylin and eosin stain. × 30.

FIG. 11. Bacilli, many with subterminal enlargements, appear in the cytoplasm of a degenerated myocardial fiber. Warthin-Starry silver stain, counterstained with hematoxylin and eosin. × 1,450.

FIG. 12. Bacilli in viable appearing myocardial fibers. Cross striations are still visible. Warthin-Starry silver stain, counterstained with hematoxylin and eosin. × 1,450.

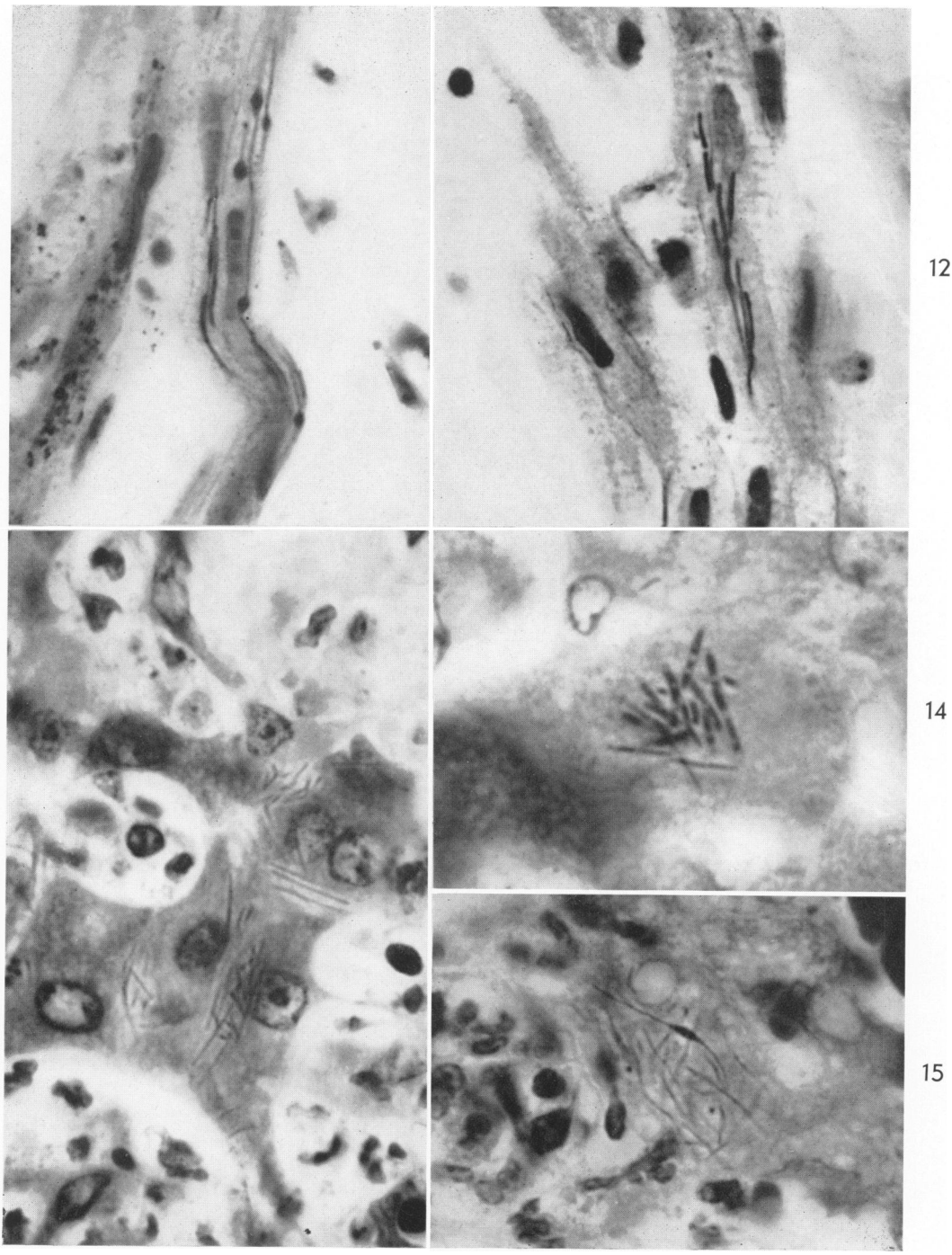

FIG. 13. Giemsa stained bacilli in the cytoplasm of formalin fixed hepatic cells. A faint vacuole or halo is observable around some of the bacilli. × 1,450.

FIGS. 14 and 15. Giemsa stained bacilli in Bouin's fixed hepatic cells showing thick banded bacilli with tapered ends and a bacillus with a fusiform subterminal swelling. × 2,000 and × 1,450 respectively.

FIGS. 16 and 17. Filamentous bacilli with bulbar subterminal enlargements and short, thick bacilli with terminal enlargements in an area of hepatic necrosis. Portions of the filamentous bacilli are out of focus. Warthin-Starry silver stain. × 1,450.

FIGS. 18 and 19. Silver impregnated bar-shaped body in the terminal enlargements of several bacilli in hepatic cells. Warthin-Starry silver stain. × 2,000.

FIG. 20. A cluster of thick bacilli with terminal enlargements in a hepatic cell containing also filamentous bacilli without enlargements. Warthin-Starry silver stain. × 1,450.

FIG. 21. Chains of irregularly spaced PAS-reactive bodies in bacilli in degenerative hepatic cells. PAS and hematoxylin stain. × 1,450.

FIG. 22. Chains of PAS-reactive bodies in bacilli in colonic smooth muscle cells in the muscularis externa. PAS and hematoxylin stain. × 1,450.

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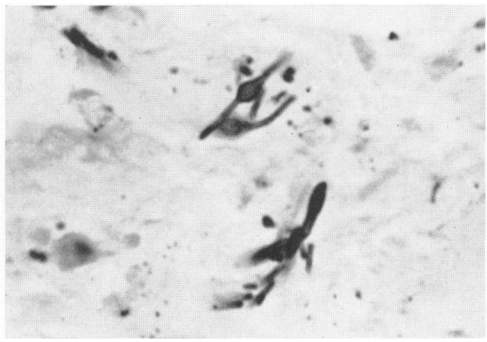

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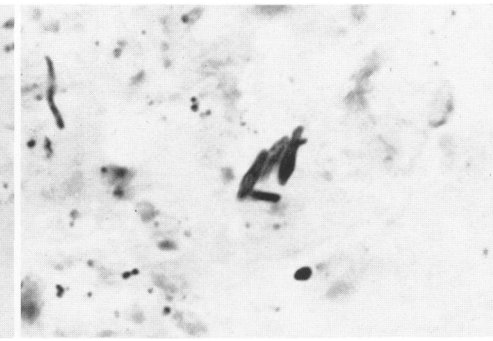

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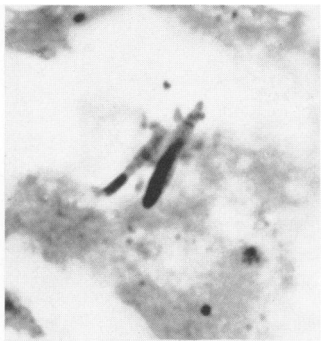

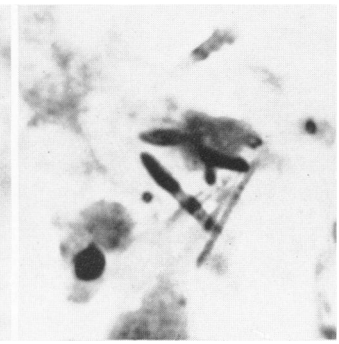

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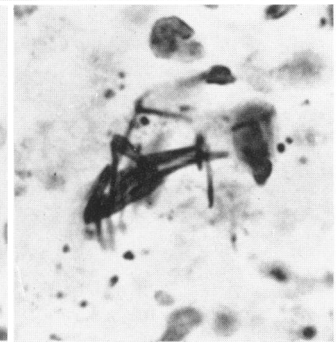

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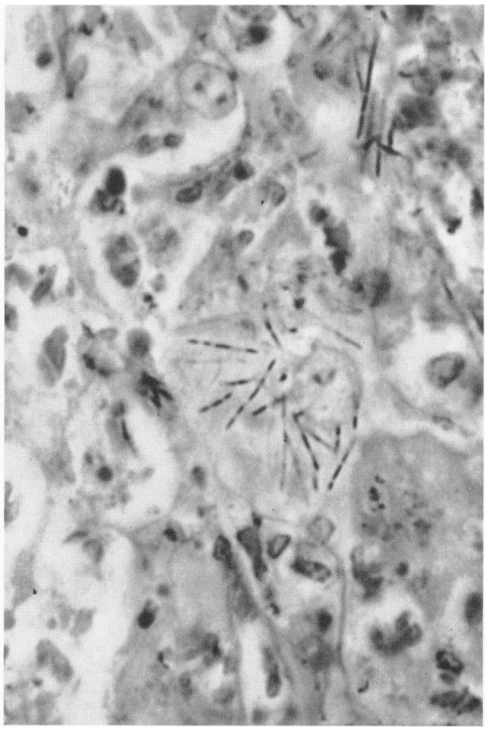

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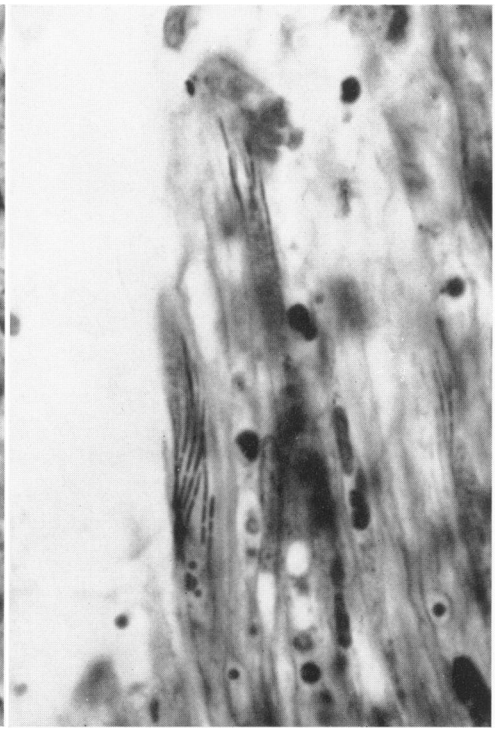

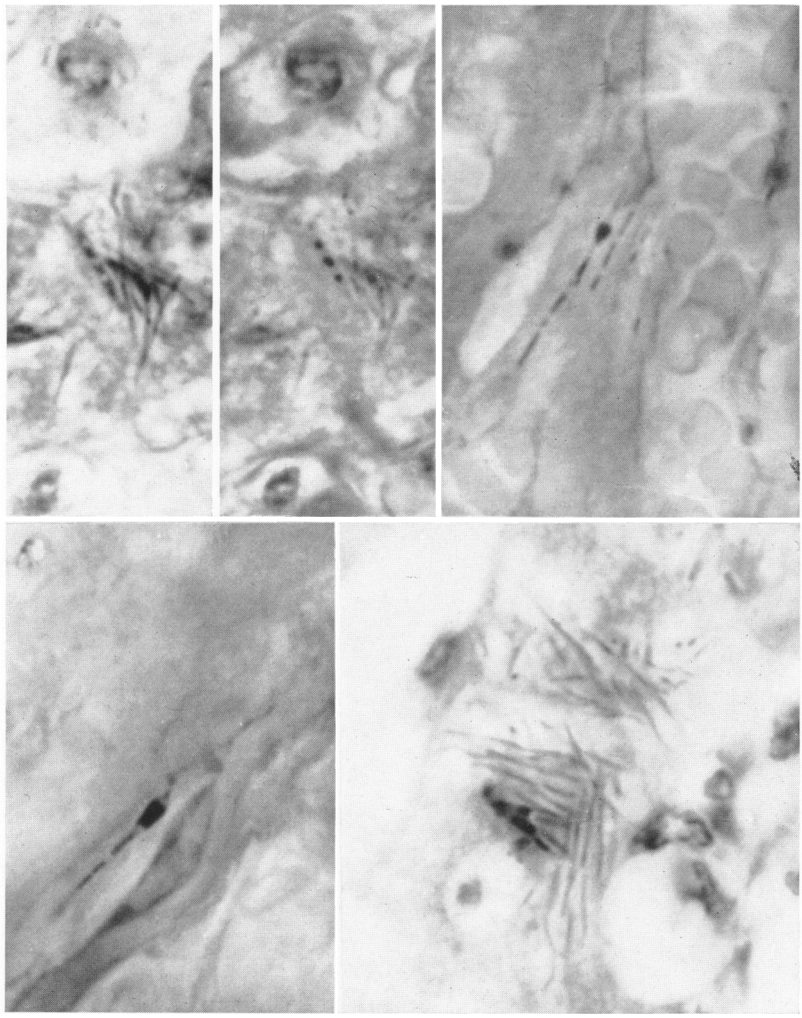

FIG. 23A. A Giemsa stained bacillus with banded appearance in a hepatic cell. × 1,450.

FIG. 23B. The bacillus shown in Figure 23A after recoloration by the PAS-reaction. The PAS-reactive bodies coincide in position with the colorless spaces of the Giemsa-banded bacillus. × 1,450.

FIGS. 24 and 25. Bacilli containing slender, rod-shaped, PAS-reactive bodies and a single wide body at the site of a subterminal enlargement. In Figure 25, the wide body appears to consist of two, thick, rod-shaped bodies located side-by-side. Approximately half of each bacillus is out of focus. They are in myocardial fibers. PAS stain. × 2,000.

FIG. 26. Two bacilli with terminal enlargements as shown with the PAS-reaction and methylene blue. Each have two large PAS-reactive bodies in their bacillary part and a small amount of PAS-reactive material on one side of the wall of the enlargement. The bar-shaped body in the center of each enlargement is colored lightly by methylene blue. The adjacent filamentous bacilli, which contain no visible PAS-reactive material, are also colored lightly by methylene blue. × 2,000.