Tyzzer's Disease

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THE DISEASE IS NAMED AFTER ERNEST TYZZER, who, in 1917, initially described this condition as a fatal epizootic diarrheal disease of Japanese waltzing mice.[1] The most obvious lesion noted at necropsy was a miliary focal necrosis of the liver. He saw bundles of a pleomorphic, gram-negative, spore-forming bacillus in apparently viable cells that bordered the areas of necrosis; he believed this was the causative agent and accordingly named it *bacillus piliformis*. He also observed the bacillus in epithelial cells associated with lesions in the lower intestinal tract. Though he was unable to culture the bacillus in any of a wide variety of cell-free media, he succeeded in transmitting the disease to other mice by injecting them intravenously with liver suspension prepared from mice that had died of the disease. Furthermore, he showed that bedding soiled by mice dying from the disease remained infectious for mice even after being stored at room temperature for 1 year. In light of present knowledge, Tyzzer's original detailed description of the disease, the causative agent and its transmission is, with minor exception, quite accurate. The occurrence of the disease in mice has been recorded subsequently in China,[2] Sweden,[3] England,[4] Japan [5] and the United States.[6]

Tyzzer's disease has been recognized as one of the major infectious diseases of mice. However, it is only during the past decade that the disease received much attention. For example, six original articles on Tyzzer's disease appeared in the literature prior to 1959, while 35 have appeared since, and 23 of these since 1965. Prior to 1965, the spontaneous or naturally occurring disease had been recognized only in laboratory mice. In that year Allen, Ganaway, Moore and Kinard[7] described a devastating epizootic of Tyzzer's disease that occurred in the rabbit colony at the National Institutes of Health (NIH). Spontaneous Tyzzer's disease has since been described in the rhesus monkey,[8] gerbil,[9–11] rat [12–14] and hamster.[11]

Despite the repeated and determined efforts of numerous investiga-

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tors, *B piliformis* has not been successfully cultured in cell-free media. The single successful claim of Kanazawa and Imai [15] remains unconfirmed. Furthermore, several factors, such as failure to form spores, loss of virulence for mice when injected by the intracerebral route, and the maintenance of viability after freeze and thaw, suggest that their culture was not *B piliformis*. Rights, Jackson and Smadel [4] thought they had successfully propagated *B piliformis* in agar-slope cultures of embryonic mouse tissue but virulence for mice was lost after the first passage. They therefore concluded that either *B piliformis* rapidly lost virulence in culture, or a symbiote necessary for producing the disease failed to grow in their cultures. Craigie,[16] in England, succeeded in isolating *B piliformis* from suspensions of diseased mouse liver in embryonated eggs inoculated by the yolk-sac route. Serially passaged yolk-sac suspensions were infectious for mice, rats and hamsters. Similarly, Ganaway, Allen and Moore [17] recently described the isolation and propagation, in embryonated eggs, of *B piliformis* from rabbits with Tyzzer's disease. Rabbits were inoculated with the 32nd yolk-sac passage and Koch's postulates were fulfilled whereby the causal relationship of *B piliformis* and Tyzzer's disease of rabbits was established.

The Causative Organism, B piliformis

The biology and natural history of *B piliformis* remains virtually unknown. Hence, its taxonomic position cannot be determined. (*Bergey's Manual of Determinative Bacteriology*, seventh edition, did not list *B piliformis*.). Wilson and Miles [18] suggested *Actinobacillus piliformis* based upon the similarity of the monilial swellings of *B piliformis* and those of *Actinobacillus muris (Streptobacillus moniliformis)*. Though the differences between these two organisms seem to outweigh this single similarity, more information is needed before judgement can be made.

The staining qualities of *B piliformis* are of considerable importance because two of the more commonly used stains in pathology—*viz*, hematoxylin and eosin (H&E) and gram stains—allow very poor visualization of the organism in tissue sections. In instances of known Tyzzer's disease, some investigators [9,10] state that the organism was not stained with H&E, while another [13] states that it was. Most investigators are in agreement that *B piliformis* stains faintly gram-negative in tissue sections. Some [1,19] have noted a tendency of the organism to stain gram-positive. We have noted this tendency to retain crystal violet in smears of infected yolk sac, using the Bartholomew tech-

nic.[20] However, when smears are decolorized with acetone or ethyl alcohol instead of propyl alcohol, the bacillus is gram-negative as it is with the Scudder or Brown and Bren technic.[7] In smears, Giemsa stains the bacillus well as a bluish-purple rod. In sections, Giemsa stains the bacillus well in cells of the liver and intestinal epithelium but stains the bacillus poorly in smooth muscle and cardiac muscle cells. In addition, methylene blue or thionin stains the bacillus in smears quickly (0.1% in distilled water for 1 minute); for this reason, they are the stains of choice in our laboratory. Thus, the bacillus is stained by strongly basic aniline dyes. It is PAS-positive and resists digestion by diastase.[7] The silver-inpregnation technics of Warthin-Starry or Levaditi are preferable to other technics for demonstrating *B piliformis* in tissue sections because the bacillus is easily recognized in the cytoplasm of all tissue cells that are known to be infected.

Pleomorphism has been reported as a characteristic feature of *B piliformis*.[1,7,16,17] Not all of the forms originally described by Tyzzer were seen by subsequent workers.[3,4,6] However, both Craigie,[16] working with mouse isolates in embryonated eggs, and Ganaway *et al*,[17] working with rabbit isolates in embryonated eggs, have confirmed Tyzzer's observations. The slender, evenly stained bacillus, 0.5 μ wide by 8–10 μ long, is the main vegetative form and is seen in large numbers within single cells during active infection (Fig 1). The rods on occasion may reach 40 μ length. Monilial subterminal swellings are often seen in rods 10 μ or more in length (Fig 2). They retain strongly basic dyes but do not retain steaming malachite green (5% in distilled water) which would be suggestive of spore formation. The beaded, banded, thickened (Fig 3) and short forms are seen less frequently. Our observations suggest that all of these forms represent transitional stages in the development of spores from the slender vegetative bacillus. The spores have not been recognized with ease in tissue sections. This might be due to staining technic, or they might not be produced to any degree in the liver, which is the tissue most commonly examined. Allen *et al*[7] recognized terminal swellings in bacilli in rabbit liver sections stained with the Warthin-Starry technic, and Fujiwara *et al*[21] demonstrated spore-like bodies in electron micrographs of infected mouse liver. Spores were easily demonstrated in stained yolk-sac smears, whether of mouse[16] or rabbit[17] origin (Fig 4).

B piliformis is motile by peritrichous flagella. Tyzzer[1] did not recognize this feature. This may have been due to the extremely unstable nature of the bacillus, a feature discussed below. Craigie,[16] using phase

microscopy, first noted motility of mouse isolates propagated in emryonated eggs. Subsequently, Fuijwara [22] reexamined sections of infected mouse liver by electron microscopy and saw peritrichous flagella. Ganaway *et al* [17] observed by phase microscopy the motility of the rabbit isolates propagated in embryonated eggs. Jonas *et al*,[12] using electron microscopy, recently demonstrated peritrichous flagella in spontaneous Tyzzer's disease of the rat.

The vegetative phase of *B piliformis* is very unstable.[16,17,23–25] *In vivo*, the organism apparently undergoes rapid lysis because intravenous passage of infective liver suspension in mice is difficult if the mouse has been dead a few hours, even when the liver is severely affected and the recipient mice are treated with cortisone.[26,27] Likewise, *in ovo*, the vegative phase undergoes rapid lysis after the embryo dies [16] so that residual infectivity in such cases is probably due to the presence of spores.[17] In yolk-sac suspensions, there is a marked loss of infectivity within 15–20 minutes at room temperature; the loss is more rapid at 37 C and infectivity is completely lost after 24 hours at 4 C.[16] The Japanese workers[23–26] have noted marked loss of the bacillus in suspensions of infected mouse liver. Their results differ from those of Craigie,[16] however. A probable explanation lies in the fact that Craigie measured loss of infectivity for embryonated eggs while the Japanese workers used the enumeration of stainable bacteria (Breed count) as a quantitative measure throughout their studies.

No method has been found thus far to stabilize the vegetative phase of *B piliformis*. Craigie[16] tested a number of factors, including pH, salt concentration, increased protein, sugar or cysteine, and anaerobic conditions. We, as well as others,[4,5,24] have found that the vegetative phase is destroyed by freezing and storage at low temperature (−70 C). Craigie [16] was able to work with a nonsporing variant by harvesting the yolk sacs of eggs before the embryo died, and freezing them within 15 minutes of harvest. This procedure, however, resulted in the loss of more than 99% of the original infectivity. We,[27a] as well as others,[5] have failed to successfully lyophilize the vegetative phase. We [27a] also have found centrifugation very harmful.[16] The bacillus is killed after heating to 45 C for 15 minutes.[16] The spores survive at 56 C for 1 hour, but are destroyed after 30 minutes at 80 C.[17] The spores survive repeated cycles of freezing and thawing.[16,17]

Clinical and Pathologic Description of the Natural Disease

Few clinical signs that clearly describe Tyzzer's disease have been noted. The sudden unexpected loss of several animals usually prompts

a closer examination of other animals, which may show depression, ruffled hair coat and varying degrees of watery diarrhea lasting up to 3 days. Emaciation is not usually seen. Indeed, it is common to note normal animals 1 day and to find dead ones the next morning with little evidence of diarrhea. Deviations from normal body temperature and blood values have not been investigated, perhaps due in part to the brief clinical course.

Under colony conditions, mortality may be very high. Tyzzer [1] lost his entire stock of Japanese waltzing mice. Allen *et al* [7] described losses as high as 50% in weanling rabbits, and Carter [9] lost up to 85% of weanling and 18% of adults in a gerbil colony. In rabbits, as in gerbils, the greatest loss occurs near the age of weaning. Loss of a single rabbit in a litter is uncommon because by the time the diagnosis of Tyzzer's disease is confirmed in the laboratory, the remainder of the litter will be dead or will die shortly thereafter. The disease occurs in adults as well but, due to less mortality, it is not as apparent as in weanling animals.

A presumptive diagnosis of Tyzzer's disease may be made at necropsy. The characteristic lesion is seen in the liver as pinpoint- to miliary-sized, pale-gray to slightly yellow spots. They are distinct, focal and scattered throughout the liver. They can be cut without resistance and would probably not be recognized macroscopically were it not for their striking light color on a dark background. At necropsy of terminal spontaneous cases, the number of spots seen on the surface of the liver varies considerably from one or two to innumerable. In the case of rabbits at least, they are often so few in number that the actual cause of death seems obscure, particularly when one considers the rapid, short course and lack of severe lesions in other organs. Varying degrees of enteritis have been noted by observers reporting on the epizootics in different species of animals. Tyzzer [1] saw little in the gastrointestinal tract of mice, except for an occasional hemorrhage in the region of the ileocecal valve. Ileitis [12] and ulcerative colitis [14] have been seen in rats. In rabbits, the most common findings are patchy mucosal necrosis in the cecum and proximal colon and edema and subperitoneal hemorrhages at the ileocecal-colic junction. Occasionally, white concentric rings of necrosis are seen in the muscular wall of the proximal colon. The mesenteric lymph nodes are swollen and juicy. No other visible lesions have been described in other organs in the peritoneal cavity. In rabbits and rats, heart lesions have been seen, consisting of slender white or gray bands that traverse diagonally the left ventricular myocardium. Thus, at

necropsy of spontaneous cases of Tyzzer's disease, the lesions occur only in the lower intestinal tract, heart, and liver. Of these, the liver lesions have been seen most frequently and are, therefore, most suggestive of Tyzzer's disease.

The definitive diagnosis of Tyzzer's disease is based upon the histologic demonstration of the causative agent, *B piliformis*, within the host cells. Once again, the liver is routinely chosen for this purpose. The organisms are seen as filamentous rods lying in bundles, randomly arranged in the cytoplasm of apparently viable hepatic cells at the border of the necrotic and normal tissue. Tyzzer [1] saw these organisms in epithelial cells of the lower intestine of mice and he attached diagnostic significance to their demonstration in mucosal smears of the cecum and colon in the absence of hepatic lesions. All investigators have remarked on the periportal location of the liver lesion, which suggests the intestinal origin of the bacillus. The detailed description of the pathology of Tyzzer's disease in rabbits [7] furnishes considerable evidence attesting to this concept. In addition to the extensive invasion of the epithelial cells of the cecum and proximal colon, the bacilli were demonstrated in the cells of the muscularis mucosa and in the circular external muscle layer. Necrosis of the smooth muscle was proximal to lymphatics laden with debris, which suggested a lymphatic as well as portal invasion. The bacilli were also found in apparently viable myocardial cells at the border of necrotic areas. The location of the bacilli in each instance, only in apparently viable cells at the border of necrotic areas, indicates a cell-to-cell spread by contact. It is worth noting once again that the bacillus appears to have an affinity for certain cells—*viz*, epithelial and smooth-muscle cells of the intestine, hepatic cells and myocardial cells. In each instance where distinct lesions were seen macroscopically (*ie*, spots in the liver, circular bands in the proximal colon and diagonal bands in the myocardium), areas of coagulation necrosis associated with the demonstration of *B piliformis* were seen microscopically. As would be expected with necrosis of epithelial cells in the intestine, varying degrees of intestinal epithelial sloughing, submucosal edema and polymorphonuclear cell invasion are seen. Hepatic lesions in various stages of healing can be seen in the same animal. This is indicated by increased invasion by polymorphonuclear cells, and subsequently, mononuclear cells; failure to demonstrate *B piliformis* within the cells at the border of the lesion; formation of multinucleated giant cells; and increased fibroplasia.

Experimental Disease

Rights, Jackson and Smadel,[4] starting with infected mouse liver from a spontaneous case of Tyzzer's disease, succeeded in maintaining *B piliformis* in over 100 serial passages by inoculating mice intracerebrally with mouse brain suspension. The mice died in 3–8 days. In such mice, *B piliformis* infection remains localized in the brain and the organism could be demonstrated in brain cells at the border of areas of liquefaction necrosis.[28,29] We,[27a] like others,[5,11,16,30] have found mice susceptible to intracerebral challenge; rats,[4,30] hamsters,[4,30] rabbits [4,30] and gerbils [30] also appear to be susceptible, while guinea pigs [28,30] and newborn chicks [28] appear to be refractory.

There was a failure to establish passage of the disease in mice by the intravenous inoculation of infected mouse liver [1,4] until Kaneko [5] demonstrated the potentiating effect of the simultaneous injection of cortisone. Using this technic, a group of Japanese workers have very actively pursued the host-parasite relationship in the mouse (over 20 publications since 1963). Because of the extreme lability of the vegetative phase of *B piliformis* and the requirement for large doses (10^4 organisms/ml) to produce lesions even with cortisone,[26] it has been necessary to maintain the organism in rapid passage; by 1965, they were working with the three-hundredth mouse passage.[24] In spite of the contributions this technic has afforded, it suffers from being laborious and confining. More importantly, rapid passage under the stress of cortisone might be expected to activate one or more of a variety of known, murine, latent infections.[31] During such rapid passage, mice die on the second or third day postinoculation. Prior to death, a bacteremia of 10^7 organisms/ml of blood is reached.[27] At this time, *B piliformis* can be demonstrated in sections of spleen, kidney, adrenal gland, mesenteric lymph nodes, lungs and heart muscle, but there is no evidence of multiplication of the bacillus in these organs and no lesions are found.[27] The only lesion observed by the Japanese workers using this technic in mice is focal hepatic necrosis that sometimes becomes confluent. Suspensions prepared from such mouse liver contain 10^9 organisms/g of liver tissue.[30] Plasma glutamic pyruvic transaminase and oxalacetic transaminase are increased; alkaline phosphatase levels are unaffected.[32,33]

A review of the numerous attempts by investigators to produce lesions of Tyzzer's disease in a variety of animal species, using various routes and tissue suspensions, would bore if not confuse the most ardent reader. The papers by Takagaki,[30] who used rapidly passaged mouse liver suspension, and Craigie,[34] who used yolk-sac passaged *B piliformis* (mouse origin), are the most informative. Briefly,

hamsters, gerbils, rats, mice and rabbits are susceptible to *B piliformis* infection. Liver lesions are produced in these species when large doses of the bacillus are given intravenously along with cortisone. Similar results may be obtained by the subcutaneous or intraperitoneal routes, but apparently with less regularity than by the intravenous route. These species also die with a necrotizing encephalitis without the aid of cortisone when they are inoculated intracerebrally. The guinea pig, dog, cat, and monkey appear to be refractory when they are challenged intravenously with the bacillus and also given cortisone. It should be recalled, however, that Niven [8] has recorded the natural infection in a rhesus monkey. Furthermore, we recently had occasion to examine a section of liver from a weanling kitten that died after a period of diarrhea. (The section was kindly supplied by Maj Robert M. Kovatch, USA, VC). The lesions were like those seen in other species of animals with Tyzzer's disease, and *B piliformis* was seen in the liver cells bordering the necrotic areas.

The administration of *B piliformis* by mouth, which is the most probable route of disease transmission from animal to animal, has produced varying results in the hands of different investigators. There are possibly many reasons for failure, such as dose size, virulence of the bacillus or resistance of the host. A more likely explanation, however, would be failure to administer a sufficient number of spores. The labile vegetative phase probably will not survive the low pH of the stomach. The shedding of spores in the excreta and their survival in the bedding for long periods at room temperature [1,7] support the hypothesis of spore ingestion for disease induction. Craigie's transmission experiments [34] likewise support this view in that a nonsporing variant given orally to mice caused no illness or lesions while the sporing strain caused death and liver lesions. The experimental disease produced in rabbits [17] given a known concentration of spores (yolk-sac passaged *B piliformis*) was similar to the natural disease in that enteric as well as liver lesions were seen in dying rabbits. It differed somewhat [27a] in that hemorrhage and necrosis were more pronounced in the distal ileum than was seen in natural cases.[7]

Predisposing Factors, Prevention and Control

Many factors have been proposed in an attempt to explain the epizootic nature of Tyzzer's disease. Among these are tumor transplantation,[1,16] genetic factors,[1,2] bacterial decomposition of soft diet,[3] diet composition,[35] irradiation,[36] cortisone administration,[5] overcrowding,[4,37] transporting,[19,37] and poor sanitation.[6,19] To this list we would add the

oral administration of sulfonamides to rabbits. This contention is based upon hindsight and unpublished observations. The disease was seen in rabbits for the first time [7] shortly after coccidiosis was eliminated from the NIH rabbit colony [38] by the process of sulfonamide prophylaxis, test and slaughter. With the view that sulfonamide treatment may have been associated with the epizootic, we gave sulfaquinoxaline-treated water to a group of 6 rabbits that had been born and raised in a room where no deaths had been observed previously. All of the rabbits on sulfa died with Tyzzer's disease within 30 days, while 20 comparable rabbits given water and similarly raised in the same room remained well. Furthermore, we have repeatedly observed that administering sulfonamides to rabbits that had been given cortisone (0.03–0.05 mg/g body weight) and placed on contaminated bedding resulted in a more predictable pattern of disease and a shortened incubation period.[17] Whether the sulfonamide causes an upset in the essential microbial flora of the cecum and lower intestine or otherwise alters host metabolism directly remains unknown.

The development of immunity to *B piliformis* infection needs considerable study. The occurrence of subclinical infection and the existence of the carrier state is assumed from the studies of Fujiwara,[25] who found complement-fixing antibody in mice from 4 of 7 colonies tested. Furthermore, weanling mice from the seropositive colonies died with Tyzzer's disease after the administration of 5 mg of cortisone, while similarly treated mice from the seronegative colonies did not die. A crude formalinized vaccine prepared from diseased mouse liver was protective for mice subsequently challenged intravenously with a lethal dose of *B piliformis,* but did not prevent liver lesions.[5,24] Mice given a sublethal dose of egg-passaged *B piliformis* were resistant to a subsequent lethal challenge dose.[34] And Fujiwara [39] recently demonstrated a protective effect of hyperimmune mouse or rabbit antisera when given to mice prior to lethal challenge.

The results of antibiotic-sensitivity testing have varied in the hands of different investigators. Craigie [34] reported a beneficial prophylactic and therapeutic effect of penicillin upon the disease in mice while Takagaki [40] tested a wide variety of antibiotics, including penicillin, and found only tetracycline beneficial. We found a partial effect of streptomycin, erythromycin, penicillin and chlortetracycline, and no apparent effect of sulfonamide or chloramphenicol upon *B piliformis* infection in embryonated eggs.[17] The brief clinical course of *B piliformis* infection, the intracellular parasitism and the development of

spores present a challenge to the prospective use of antibiotics or chemotherapeutic agents in the treatment of the disease. Their greater value might be in the prophylactic treatment of valuable contact animals.

At the present time, there are no sound recommendations for the elimination of the disease because this aspect has not been studied. At least in the case of mice and rats, the use of progeny from cesarean-derived animals should be adequate, provided their environment is protected from extraneous contamination. While some of the other species of laboratory animals have been produced under these conditions, these animals are not readily available. Theoretically, at least, if the environment is kept free of spores and cage-to-cage spread is controlled (*eg*, through the use of several available types of filter cage covers) the epizootic can be controlled. The possible carrier state presents a problem, however, and if such animals are stressed as previously mentioned, they may shed large numbers of spores into the environment and thus permit transmission to other animals.

Interference with Interpretation of Experimental Data

It is impossible to assess the degree to which *B piliformis* infection has interfered with the interpretation of experimental data. Not only has the disease probably not been recognized in many instances when it was present, but negative aspects of study are often not published. On the other hand, it is ironic that Tyzzer [1] noticed this disease for the first time during the course of tumor transplantation studies in mice. Thus, knowledge of the disease had its genesis in cancer research, an area of maximum effort today, and in the mouse, the most widely used laboratory animal. Attesting to this problem in cancer research, the British [41] suggest that Tyzzer's disease has been responsible for more ruined cancer research than any other mouse disease. Niven [8] alluded to the interference of Tyzzer's disease in older mice during tumor transplantation studies, long-term experiments with chemical carcinogens and immunologic research conducted after thymectomy. Tuffery [36] observed a high death rate (47%) due to Tyzzer's disease in irradiated mice while none of the nonirradiated mice died. This suggested not only an activating effect of irradiation but a high carrier rate as well. This activating effect of irradiation was subsequently confirmed and was found to be similar to that of cortisone.[30] The effect of cortisone upon the induction of fatal *B piliformis* infection is well documented, as previously noted. The wide use of cortisone and other immunosuppressants probably causes more *B piliformis* infection than is apparent in the literature.

Perspective

In spite of all that has been said, we know little about *B piliformis* and Tyzzer's disease. The organism is a gram-negative, motile, spore-forming, obligate intracellular parasite. These collective characteristics are unlike those of any previously described microorganism. It appears to kill a wide variety of animal species with a high attack rate. Its occurrence in species other than the mouse has been detected only during the past 5 years. Why? Though the answer remains unknown, we suspect that increased awareness of and surveillance for the disease offers the most logical explanation. If this is true, then occurrence of the disease is more frequent and in a wider range of animal species than was previously believed. *B piliformis* is elusive, not only because of its poor staining qualities in tissue sections, but because the organism evades being cultured in bacteriologic media while having the appearance of a bacterium.

Possibly the greatest single advance is the recent isolation and propagation of the agent in embryonated eggs, which has resulted in a better understanding of the biology of the organism. Once again, however, the elusive nature of *B piliformis* defies the investigator because the vegetative organism undergoes rapid lysis outside the host cell. This characteristic of *B piliformis* is probably responsible for many of the failures encountered thus far. There is a need to propagate the organism in cell cultures because the use of embryonated eggs is cumbersome and does not permit a detailed study of the growth and maturation processes. But this achievement seems secondary to the knowledge of how to stabilize and preserve the vegetative phase.

There is a strong temptation to speculate on the role of *B piliformis* in the intestinal infections of unknown cause that occur in a variety of animal species. Perhaps it is better to simply state that we have much to learn about *B piliformis* infections.

Summary

Tyzzer's disease occurs as an acute, fatal, epizootic, diarrheal disease of the mouse, rat, hamster, gerbil, rabbit, cat and subhuman primate. The following lesions are seen at necropsy: hemorrhage, necrosis and edema in the lower intestinal tract; scattered white-to-yellow miliary spots in the liver and, occasionally, a white narrow band that traverses the ventricular myocardium. The causative organism, *Bacillus piliformis* (Tyzzer), is demonstrated histologically as bundles of rods, 0.5 × 8–10 μ, in the cytoplasm of apparently viable cells bordering areas of coagulation necrosis in the intestinal epithelium,

smooth muscle of the muscularis mucosa and muscularis externa, liver and myocardium. Unclassified as yet, the bacillus is further characterized by the following: failure to grow in cell-free media, growth in the yolk-sac epithelium of the embryonated hen's egg, gram-negative reaction, affiinity for strongly basic aniline dyes, motility by peritrichous flagella, pleomorphism, spore forming, instability of the vegetative phase and sensitivity to certain antibiotics. A probable explanation for the recent recognition of the disease in a variety of animal species lies in an increased awareness of the disease and the realization that the bacillus is poorly demonstrated in tissue sections with hematoxylin and eosin or gram stains. Silver impregnation is the method of choice. Of the many predisposing factors that have been suggested, noteworthy are the buildup of spores in the environment and the alteration of immune mechanisms that occur after thymectomy, cortisone administration and irradiation.

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[Illustrations follow]

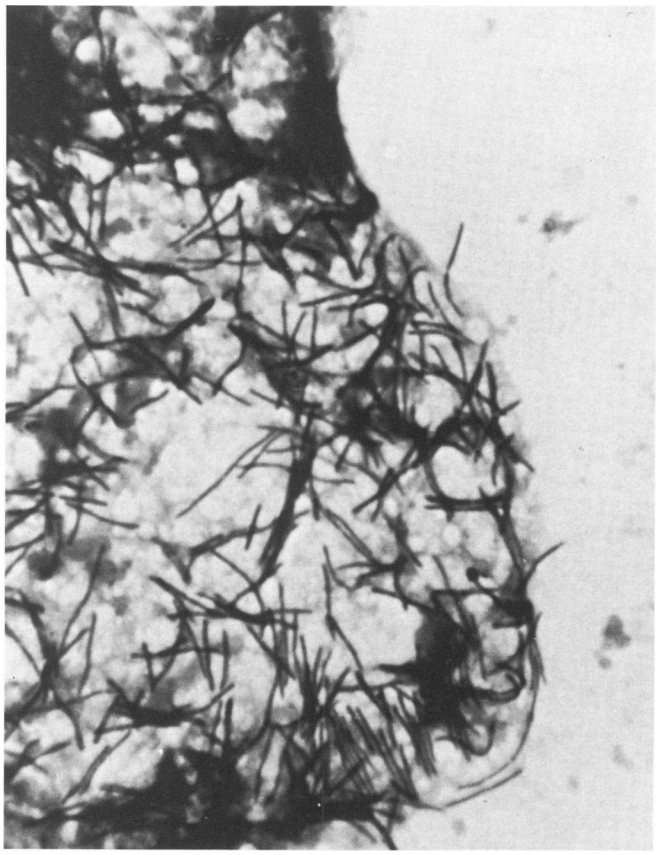

Fig 1 — Predominant vegetative form af *B piliformis* seen in epithelial cells of yolk-sac smear. Not all of the bacilli are in focus. One thickened bacillus appears near the upper center (safranin stain, × 1650).

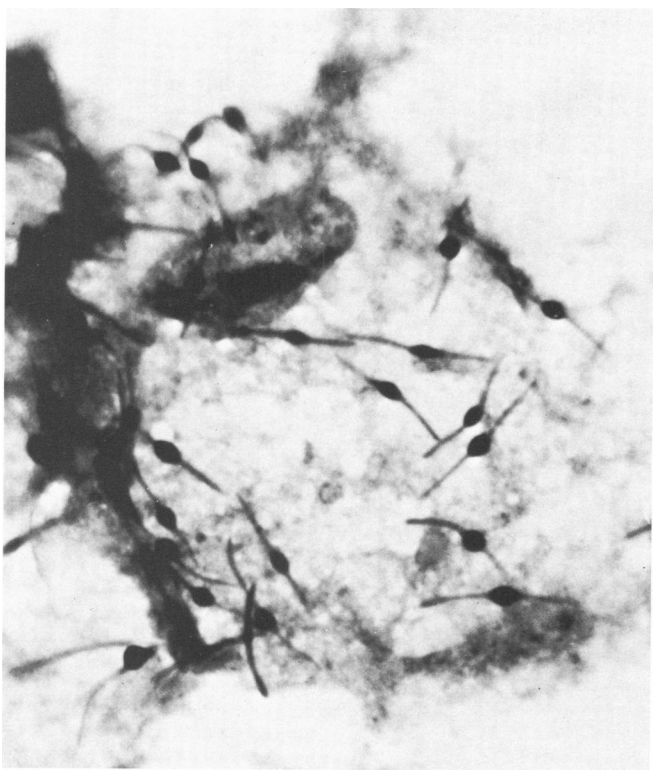

Fig 2 — Monilial swellings of *B piliformis* in an epithelial cell of a yolk-sac smear (safranin stain, × 1650).

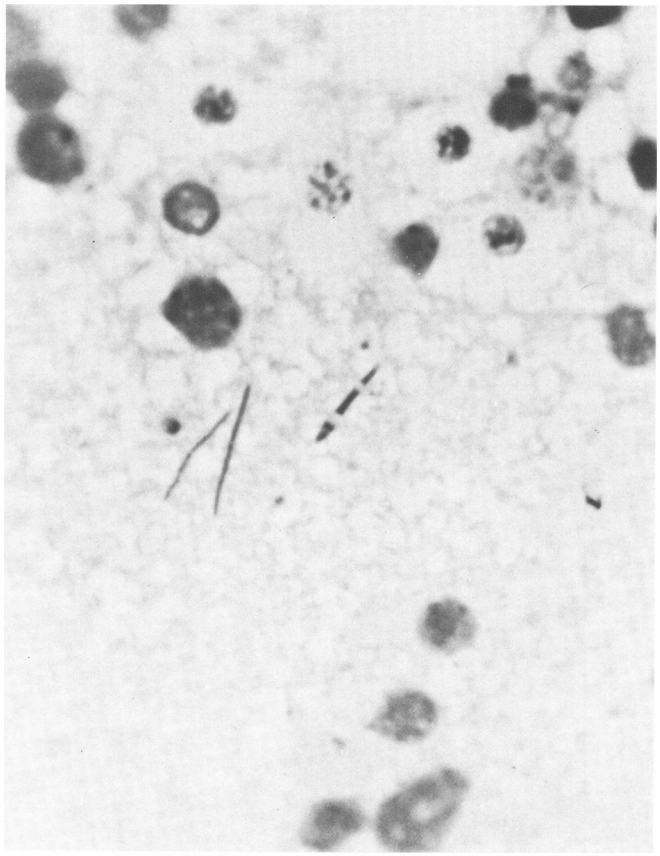

Fig 3—Less often seen form of *B piliformis* in yolk-sac smear. The bacillus appears thickened, banded and tapered at the ends (safranin stain, × 1650).

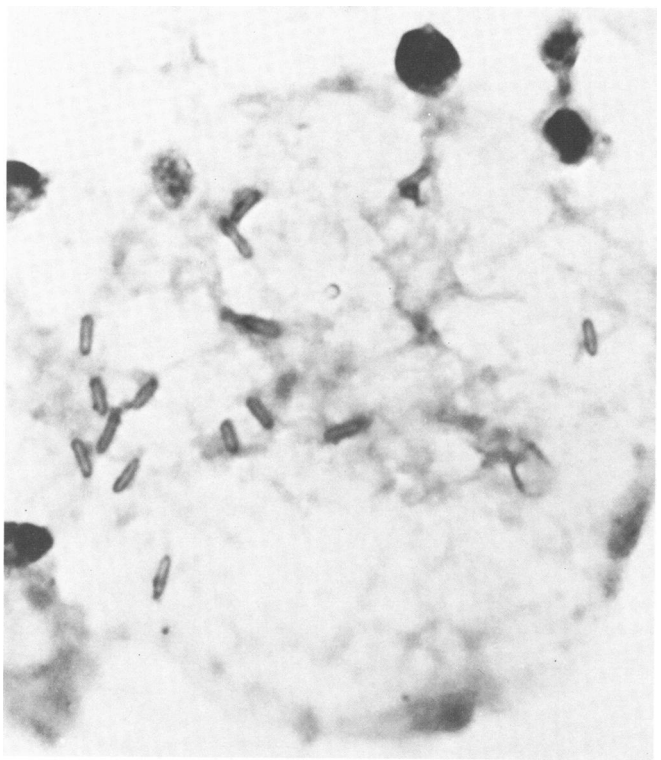

Fig 4—Spores of *B piliformis* in an epithelial cell of a yolk-sac smear (spore stain, × 1650).