A FATAL DISEASE OF THE JAPANESE WALTZING MOUSE CAUSED BY A SPORE-BEARING BACILLUS (*Bacillus piliformis*, N. SP.).*

ERNEST EDWARD TYZZER.

(*Department of Comparative Pathology, Medical School of Harvard University.*)

In the course of a series of experiments involving the transplantation of certain mouse tumors into Japanese waltzing, common, and hybrid mice, a disease appeared which soon became epidemic among the Japanese waltzing mice and certain types of hybrids. This is characterized by multiple lesions of the liver, associated with a bacillus which is distributed chiefly within living liver cells. The spores of this bacillus occur in the liver lesions where they may develop prior to the death of the mouse, but are found in greater numbers in the intestinal tract. On the appearance of a single case of the disease in a cage, other mice either living in the latter at the time, or subsequently placed in it, almost invariably succumbed to the same infection. The disease had spread to such an extent before its serious nature was recognized that it was only by the most radical measures that the loss of several valuable strains of transplantable tumors was prevented. These were saved by being transferred to newly purchased Japanese waltzing mice, which were placed in sterilized cages, fed upon sterilized food, and kept isolated from other mice.

The Japanese waltzing mice, with the exception of a small number reared in the laboratory, had been purchased from a breeder who, notwithstanding the susceptibility of this variety to disease, had never suffered any appreciable loss from this cause. These mice after purchase had been kept in a room at a distance from the quarters of the common tame mice, many hundreds of which were constantly on hand. However, in the course of cross-breeding the two varieties, the mated common and waltzing mice, together with their

* Received for publication Sept. 14, 1917.

offspring, were in some instances quartered with the common mice and in others with the waltzing mice. Considerable numbers of hybrids were also transferred from time to time from the quarters of the common mice to those of the Japanese waltzing mice. Thus, while the latter animals were kept somewhat isolated, the occasional introduction of hybrids from the quarters of the stock mice might readily furnish opportunity for the transmission of certain parasitic organisms. The possibility that this disease may have been derived from some other source, as, for example, from other laboratory animals or from grain contaminated by wild mice previous to purchase, cannot be excluded, but there is no evidence indicating such an origin.

Epidemiology. — The multiple liver lesions characteristic of the disease were first noted in a hybrid of the second filial generation (F_2) dying on Jan. 26, 1915. The disease next appeared one month later in hybrids $(F_1 \times J.\ w.)^1$ obtained by back-crossing F_1 hybrids with the Japanese waltzing mouse. On March 3d a second generation hybrid $(F_1 \times J.\ w.)^2$ from back-crossing succumbed, and on March 8th the disease was first noted in a Japanese waltzing mouse. There is thus, in the earlier occurrence of the disease among hybrids, circumstantial evidence of its having been derived from the common mice with which they were for a time associated. Following its appearance among the waltzing mice the entire stock of these animals succumbed to this scourge, and those newly purchased promptly contracted the infection until radical preventive measures were adopted. Seventy-nine Japanese waltzing mice were lost in this epidemic, the last one dying on June 24, 1915. The isolation of newly purchased Japanese waltzing mice in sterilized cages, and the sterilization of food and water served to eradicate the disease so that no case appeared subsequently among them, except in those intentionally infected, until January, 1917, when it reappeared in a single cage. For several months prior to its reappearance, both the experimentally infected and normal mice were kept in a small room and,

although precautions were taken to prevent any interchange of material between the two, neither cages nor food were sterilized. Under such conditions the reappearance of the disease may be accounted for by the aerial transportation of spores to the food or cages of normal mice during sweeping and cleaning. In the meantime the continued presence of this disease in the quarters of the common stock mice is shown by its appearance in the months of July and October of 1916 in hybrids that had been kept with the common mice. Although during the early epidemic the disease appeared to be invariably fatal to Japanese waltzing mice, common mice living under the same or more unfavorable conditions remained apparently healthy. Up to the present time it has been observed in only two common mice, notwithstanding that many hundreds of these are kept constantly in stock.

From the extreme rarity of its occurrence in common mice together with its continued incidence in hybrids kept in the same quarters with the former, it is necessary to consider the possibility of the common tame mouse serving as the carrier of an organism which is distinctly pathogenic to the Japanese waltzing mouse and certain types of its hybrids.

Pathology. — The post-mortem findings in this disease are remarkably constant. At the time of death there is no notable emaciation, but the fur about the tail is usually soiled or stained yellowish with watery or slimy fecal discharges. There is an unusually marked post-mortem rigidity, as the result of which the body remains rounded and the thorax fully expanded. The liver alone of the internal organs presents visible lesions which somewhat resemble miliary tubercles. They appear as small rounded nodules, which vary in size from a small fraction of a millimeter to two and occasionally to two and a half millimeters in diameter. They are usually opalescent and of a pale gray color, in some instances more opaque and tinged with yellow. Some lesions show an opaque grayish, others a dark-brownish central point, with an opalescent peripheral zone. In animals

which resist the disease for a longer period than usual, the lesions may present a red central portion evidently due to a growth of new blood vessels in the organization of the necrotic tissue. These variations probably indicate different stages in the development of the lesions and correspond with differences seen microscopically. The lesions in the acute stage are of firmer consistence than the normal liver tissue, but are readily crushed. Small lesions are not especially dry and their entire substance is readily spread in making smear preparations, but the larger lesions are usually dry and of caseous consistence. There is considerable variation in the number present; some animals showing only one or two, others, innumerable lesions. When numerous they are generally distributed throughout the entire liver and are for the most part discrete, only an occasional exceptionally large nodule representing the confluence of several lesions. The liver is considerably enlarged in severe cases. No jaundice has been noted. Intestinal infection without involvement of the liver also occurs. The spleen is not notably enlarged and all other organs appear normal.

On histological examination the liver lesions are found to consist of necrotic tissue showing extensive infiltration with polymorphonuclear leucocytes, which is more marked in certain areas, but especially near the surface of the nodules. The most distinctive feature of the lesion is the presence at its periphery of long slender bacilli situated within the living liver cells.

Liver lesions having a somewhat similar appearance have been observed in another infectious disease of the mouse. Multiple nodules of necrotic tissues were observed by the author many years previously in an epidemic occurring in common mice. This was evidently due to a short thick bacillus, which was found growing in masses in the interior of the necrotic nodules. It was thought that this disease might be mouse typhus, but no attempt was made to definitely identify the organism. Both the microscopic appearance of the lesions and the nature and distribution of the bacillus present serve to distinguish this infection from the waltzing mouse disease.

These organisms lie more or less parallel to one another in the living cells and are not as a rule apparent in the

necrotic tissue, although they may occasionally be seen within phagocytic cells in the interior of the lesion. They are thus for the most part distributed within the liver cells, either in a more or less continuous layer, or in larger or smaller groups just outside the line of demarcation, which in these lesions is very sharply drawn between the living and the necrotic tissue. The spores as well as the vegetative forms of the bacillus are apparent in both stained sections and smears. In some cases with extensive liver involvement no bacilli have been found in smears of the bile, but in others slender bacilli and spores have been found in the bile and in the epithelium of the gall-bladder, of the common and of the hepatic duct.

The earliest lesions are represented by minute collections of polymorphonuclear leucocytes, situated adjacent to liver cells invaded by these bacilli. The lesions are generally developed in close proximity to the portal veins, indicating an embolic distribution of bacilli from an intestinal source. The larger discrete nodules thus very frequently show, in section, a distended portal vein extending axially with reference to the nodule. The lesions vary somewhat in character, and, while some show a trace of the topography of the liver in the necrotic area, others do not. The interior of the lesion usually shows a reticulum of fibrinoid material enmeshed, in which are polymorphonuclear leucocytes and cell débris. Toward the periphery this reticulum may be continuous, with columns of necrotic liver cells. It in some instances appears as an open network, with large, clear spaces, and with relatively few leucocytes, but is frequently more compact. Some lesions show toward the periphery an irregular zone of necrotic liver cells, which appear hyaline and are intensely acidophilic in their staining reaction. In other lesions this hyaline necrosis is not in evidence, but the liver cells appear to melt away in the presence of the bacilli and polymorphonuclear leucocytes. The latter are always accumulated in greater numbers at the periphery of the lesion. There may be cloudy swelling of the liver parenchyma apart from the lesions, but there rarely is evidence of

more advanced degeneration, and then only when the organ is extensively involved. It is probable that the more marked changes are based on circulatory interference during the development of the lesions rather than on the production of toxic substances within the latter. Collections of vacuolated macrophages are found distributed independently of the lesions in certain livers.

In older lesions newly-formed connective tissue appears. This may grow in from the periphery, and is frequently found growing from the radially directed portal canal. It consists of very cellular connective tissue, through which are distributed polynuclear leucocytes, lymphoid and plasma cells, and endothelial phagocytes in varying numbers. During the process of repair the lesion may be replaced by cellular connective tissue in such amount as to constitute a nodule of considerable size, and a centrally situated sequestrum of necrotic material, associated with large numbers of macrophages, is frequently found. This sequestrum of necrotic material probably accounts for the different color of the central portion observed in certain lesions, although the red central areas in late lesions are evidently due to newly-formed blood vessels. In other cases the necrotic material becomes absorbed, with slight increase in connective tissue, so that there is little more than a loss of substance to indicate the distribution of former lesions. Various stages of the disease process, from active extension of the infection to quite advanced repair, may be present in a single liver.

Thus while the lesions are in general of rather firm consistence and resemble miliary tubercles in their gross appearance, they are to be considered from their microscopic appearance as more of the nature of miliary abscesses.

The alimentary tract generally shows no visible lesions and no marked reddening. In one instance a small hemorrhagic area was noted in the region of the ileo-cecal valve, and in one instance there was a minute hemorrhage in the wall of the stomach.

Sections of the cecum and the first portion of the large intestine show the same slender bacillus growing in the

epithelium. Both the vegetative forms and spores occur in great numbers in the epithelium of the more superficial portions of the mucosa, but are also found in considerable numbers in the depths of the glands. These bacilli infecting the epithelial cells are by their morphology readily distinguished in section from the coarser fusiform bacilli which normally occur in the lumina of the glands. The former appear stiff and hair-like and lie more or less parallel within the cells. They are each surrounded by a minute clear space in the cytoplasm and, while some are seen in longitudinal section as slender rods, others in cross-section appear as small, deeply stained dots, situated at regular intervals. They are not found in masses as are many other bacteria, but are isolated from one another, each lying in a separate space within the cytoplasm of the epithelial cells. They appear to grow from one cell into another and it is possible occasionally to follow filaments of considerable length. It is evident that the organism becomes shorter and thicker preparatory to spore formation. Both the bacilli and their spores are found in endothelial phagocytes and polymorphonuclear leucocytes beneath the epithelium, also in small foci in the interglandular connective tissue, and in several instances have been found growing in the walls of blood vessels in the mucosa. Free bacilli have been found in considerable numbers in small vessels, presumably lymphatics, accompanying small blood vessels. There are usually no bacilli in the mucus of the secreting cells, but they are frequently found in empty goblet cells. The epithelium of the small intestine is also infected by this bacillus, but the sections examined indicate that it does not grow here as profusely as in the large intestine. The epithelium covering the tips of the villi is quite extensively invaded, but the bacilli are not so frequently found infecting the glandular epithelium as in the large intestine.

Smears from the fecal discharge show, intermingled with the other intestinal forms, great numbers of slender bacilli and spores morphologically similar to those found in the epithelium, but, although the spores are quite characteristic, the positive identification of the specific organism in its various

forms, under such conditions, is too difficult to attempt. The presence of the slender bacilli and spores within epithelial cells in smears of the mucosa of the large intestine or cecum makes possible the diagnosis of infection.

The reaction of the intestinal tissues to the infection is almost negligible. Even severely infected epithelium may show slight evidence of degeneration. In certain animals, however, masses of hyaline material occur in the epithelium and there are foci of degeneration in certain glands. Minute collections of polymorphonuclear leucocytes are occasionally present beneath the epithelium, and in several instances collections of leucocytes have been found in a gland, the epithelium of which had been partially destroyed. Microscopic abscesses may thus be present and in a few instances the destruction of several adjacent glands has resulted in the formation of minute defects in the mucosa.

Bacillus piliformis, n. sp. — Since the organism constantly associated with this disease does not appear to conform in its characteristics with any bacillus heretofore described, it is regarded as a new species, and on account of its slender form and stiff appearance the name *Bacillus piliformis* is proposed. Although it has not yet been successfully isolated in pure culture, its morphological characteristics are such that it is readily recognized, and its relationship to the lesions leaves slight ground for doubting its etiological significance. Its peculiarity in invading epithelium, its distribution chiefly in the intestine and liver, with the production of visible lesions only in the latter, and the provision for the dissemination of spores during the life of its host, all assist in differentiating it from other pathogenic organisms.

B. piliformis is a slender, Gram-negative bacillus which produces spores. No motility has been noted, although repeated observations have been made. This bacillus shows a considerable degree of pleomorphism, and in smears from the liver lesions it varies greatly in length, in thickness, and in its affinity for bacterial stains. In both smear and section it is noted that certain forms stain more uniformly and

intensely, while others stain unevenly and less intensely. The former are quite uniform in thickness (.5 micron), but vary from four to twenty-five microns in length. The latter vary greatly in diameter so that some appear slender and hair-like, others thick and coarse. In such forms the deeply stained material is arranged in granules at regular intervals throughout the length of the organisms, so that they have a banded appearance. The deeply stained granules are small as compared with the faintly stained bands, except in over-stained preparations, in which they appear larger. With the Romanowski stains these granules stain an intense red and the remainder of organism bluish. The frequent arrangement of the deeply stained material in paired granules, as well as its affinity for nuclear dyes, suggests that it is of the nature of chromatin. A large proportion of the organisms taper slightly toward the extremities and this tendency is pronounced in the banded bacilli, the larger of which resemble slightly the fusiform bacilli of the alimentary tract. Of the intensely stained forms, the longer rods taper slightly, but the short rods have blunt, slightly rounded ends. In fresh material and in stained sections the organisms are generally straight, but in smear preparations the longer forms are frequently bent so that they are either looped or wavy. This observation indicates that the rods are soft and pliable. Certain organisms also appear very broad in stained smears, as though flattened in the course of their preparation. Long rods not infrequently show a centrally situated fusiform swelling, which does not retain the stain after the manner of the spore. Such possibly represent involution forms of the bacillus, although it is quite suggestive of spore formation. Pairs of organisms attached end to end are present and occasionally a long unsegmented thread, but chains are not found. The great majority occur singly and when grouped have their long axes extending in the same general direction. Within the epithelium, and especially that of the intestine, the organisms in general are found extending lengthwise in the cells. It appears that following transverse fission within the cells the resulting rods lengthen and grow past each

other, so that they come to lie parallel. The organism never occurs in clumps, but each rod lies in a separate space in the cytoplasm of the living cell — indicating a high degree of adaptation.

The spores are long, ellipsoidal in form, and are sub-terminal in their development. They vary from three to four microns in length, the average length being 3.3 microns, and from 1.1 to 1.8 microns in thickness. The portion left unstained by ordinary dyes is cylindrical. With spore stains (carbol fuchsin followed by two per cent sulphuric acid and counter stained with Loeffler's alkaline methylene blue), there is a slender, cylindrical, centrally situated, stained portion surrounded by a clear zone and this in turn enclosed by a bulging outer layer of varying thickness which takes the counterstain (see Fig. 21). The spores develop from the banded forms, a series of which is shown (in Figs. 10 to 16) in the probable order of their development. The organisms become greatly thickened preliminary to spore formation, and that division into shorter segments takes place is indicated by the frequent occurrence of spores in pairs. The portion of the organism which becomes transformed into the spore at first stains deeply, but later, probably after the formation of a capsule, is left unstained. There is frequently a deeply stained granule near the end of the bacillus opposite to that in which the spore is developing, but the arrangement of such material is not sufficiently regular to enable one to determine successive changes in its distribution or to indicate its nature. The remains of the rod from which the spore has developed persists for a time and is found extending from one end of the latter, but eventually disappears. The mature spores frequently show a slightly projecting knob at either end.

The organism in general colors a purplish tint with Loeffler's blue, is non-acid fast and Gram-negative, except that in the banded forms the granules may retain the violet stain. McJunkin's[1] modification of the Giemsa stain colors the granules deep red in marked color-contrast to the remainder of the bacillus, which is tinted bluish. This

method also makes the rods appear rather thicker than the common bacterial stains.

B. piliformis shows certain features in common with *B. (Leptothrix?) pyogenes filiformis* Flexner,[2] and in fact appears to be as closely related to this as to any known species.

Certain of its forms are similarly regularly banded, but the deeply stained portions are considerably narrower than the faintly stained bands. Flexner's bacillus was cultivated with difficulty after failure to grow on ordinary media. No spores were noted and the bacillus grew in necrotic tissue, rather than in living cells. It furthermore was pathogenic to rabbits and not to mice.

Being unacquainted with Dr. Flexner's paper, the author at first considered the name *filiformis*, but subsequently rejected this in favor of *piliformis*. The similarity in nomenclature was therefore unintentional, but under the circumstances apparently appropriate.

Cultures. — Repeated attempts to cultivate this organism in pure culture have failed, although on one occasion it has possibly grown for a time in symbiosis with other bacteria. On at least seventeen different occasions the diseased liver has been planted on various sorts of media, but the majority have shown no growth under either anaerobic or aerobic conditions, while the remainder have shown contaminating organisms frequently met with in mice. The following media have been tried in these attempts — plain agar, glycerine agar, serum agar, sugar-free agar, agar with liver extract, blood agar, bouillon, sugar-free bouillon, bouillon with liver extract, bouillon with intestinal extract, liver extract, ascitic fluid, and ascitic fluid with tissue with or without oil, gelatin, N.N.N. medium, Dorset's egg medium, and litmus milk with sterile tissue. In many instances large pieces of diseased liver were used in inoculating the tubes, so that the lesions were plainly in evidence in the culture tubes, but there was no growth, even in the vicinity of the lesions.

In the single instance in which a bacillus presenting the morphological feature of this one grew for a time with other bacteria, a slant of sugar-free agar showed in the water of

condensation after three days' incubation under anaerobic conditions, a great number of delicate-curved bacilli with tapering ends, and a few larger bacilli similar to those found in liver lesions. Sub-cultures were made and incubated under anaerobic conditions, together with the original tube. Three days later the delicate-curved organisms were practically replaced by a small streptococcus, and the bacillus similar to that found in smears from the lesions continued to increase. The sub-cultures showed only streptococcus, and the bacillus having the appearance of *B. piliformis*. Repeated attempts were made to separate the latter by plating, but only the streptococcus grew. A variety of media was used, but the bacillus grew only on sugar-free agar in the presence of the streptococcus. Under these conditions the bacillus grew feebly and had practically died out by the fourth sub-culture. What appeared to be atypical spore formation (Fig. 6) was noted occasionally and a large proportion of the bacilli stained poorly as the cultures became older.

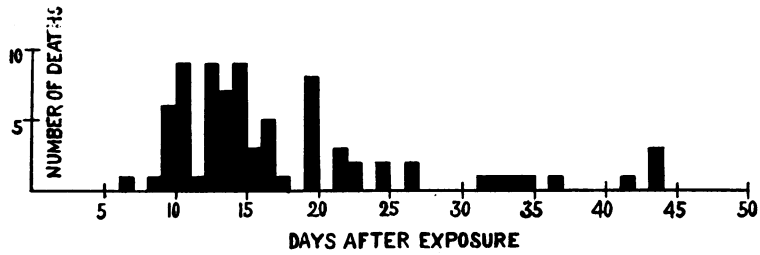
These attempts at cultivation indicate that this bacterium requires conditions for its growth not furnished by the media commonly used in bacteriological work.

Distribution. — It was thought possible that *B. piliformis* might prove to be a frequent parasite of the common mouse. Smears from the mucosa of the small intestine, the cecum, and the colon of a number of wild and tame mice from different sources have thus been examined. Long terminal spores have been occasionally found in the contents of the large intestine in both tame and wild mice, but, since somewhat similar spores are found developing in different types of intestinal bacilli, positive identification on morphological features alone has not been attempted. Neither spores nor bacilli were found in the epithelial cells in either smears or stained sections of the intestine of these mice. A large number of stained sections of the large and small intestine of laboratory mice used during the last twelve years have been reëxamined, but in no instance was the epithelium found to be infected. Thus while it is not proved that *B. piliformis*

is not generally distributed in the mouse population, all the available evidence indicates that it is not. It appears more probable that it only flourishes where great numbers of mice are kept in crowded quarters.

Symptoms. — In severe cases the infected mice usually show no evidence of the disease up to the last forty-eight or twenty-four hours preceding death. They may then totter somewhat in moving about owing to weakness, present a staring of the fur and show diarrhea with more or less watery fecal discharges. Either for cleanliness or on account of local irritation, the affected animals lick away the discharge from the region of the anal opening. A notable perversion of appetite is shown in the persistence of younger animals coming down with the disease in licking the fecal discharges from one another. It is thus not only through food contamination associated with the waltzing habit, but also through the vices encountered in young Japanese waltzing mice, that the transmission and undue multiplication of intestinal parasites is favored. From observations on infected Japanese waltzing mice it is evident that their tendency to diarrhea results in the ingestion of the specific bacilli in increasingly great doses, so that in such cases it is not remarkable that the animal is finally overwhelmed. On the other hand, adult animals in which the disease is running a fatal course may show no evidence of diarrhea until just prior to death.

The true period of incubation has not been definitely determined since there are no obvious symptoms marking the onset of the disease. The following chart will show the distribution of deaths with reference to time after the first opportunity for infection.



It is evident that death occurs most frequently in from ten to sixteen days, but not infrequently later up to twenty-seven days, after exposure in a contaminated cage or after the ingestion of infective material. The late occurrence of death is probably conditioned by different factors, such as smaller doses of the infectious agent or a reaction of the host unfavorable to the growth or dissemination of the latter. The influence of dosage is shown in the marked retardation in the course of the disease, which has been effected through the isolation of the infected mice from one another in clean battery jars. The confinement of a number of infected animals together in a single compartment would tend to equalize the environment for all, while isolation would furnish certain animals at least with fewer organisms through which they could reinfect themselves. In fact, certain infected mice lived for over forty days under these conditions, and death may have been due to other conditions, since the lesions showed evidence of repair. Others when killed at this time showed healing lesions. Complete recovery occurred in one mouse which had developed liver lesions and had shown evidences of diarrhea at the time when others of the same lot were dying of the disease.

Variation in the character of this disease, as it occurs in Japanese waltzing mice, is probably due to changes in the resistance of these animals with age and to conditions which increase or decrease the amount of infectious material ingested. The acute form occurs frequently in young non-resistant animals, which succumb early with marked intestinal symptoms. There is severe diarrhea for several days before death, when there may be reddening of the cecum and microscopic evidence of extensive intestinal infection, but slight involvement of the liver. The latter may present only one or two small lesions in its surface, and, in some instances of intestinal infection, no liver lesions have been found. The more characteristic form of the disease occurs in mature mice, which apparently resist extensive intestinal invasion, but eventually die from the involvement of the liver. Diarrhea occurs in some cases for a day or two prior to death, but is absent in others. Death does not occur so early as in the acute form; the liver lesions are well

developed, and usually in considerable number. The milder and more chronic type of the disease, such as occurs in mice which after exposure are kept individually isolated in clean cages, may be without notable symptoms. Death occurs considerably later than in the preceding types, and in rare instances recovery may occur. The liver lesions are few in number, but usually relatively large. The process of repair is well advanced in some animals which succumb. Except in instances of unusual individual resistance, conditions which diminish the dosage of the infecting organism serve as a basis for the mild form of the disease.

Diagnosis may be made during life, especially in young animals, by removing the fur from the abdomen. The ventral surface of the liver is, in bright daylight, then visible through the semi-translucent abdominal wall, especially if the body of the mouse is fully extended, and any large lesions which may occur in this area may be seen oscillating up and down with the respiratory movement of the diaphragm. The identification of *B. piliformis*, either in the liver lesions or in the intestinal epithelium, is essential for absolute diagnosis.

EXPERIMENTAL INFECTION.

Contaminative method. — The disease may be produced by placing the Japanese waltzing mice in uncleaned cages in which others have succumbed. For example, seven mice were placed on May 15, 1915, in a cage in which the disease had occurred. Two died thirteen days, two fourteen days, and three fifteen days later, and all showed the characteristic lesions of the liver. This procedure has been frequently repeated with similar results. Experimental infection of the waltzing mouse by exposure, by the ingestion of diseased liver, and of intestinal contents is shown in the following experiment, but the results of the subcutaneous inoculations are vitiated by the unintentional transference of the infection to this, as well as to the control group. In this experiment the susceptibility of common mice, guinea-pigs, and rabbits to the disease was also tested.

EXPERIMENT I.

The twenty-five Japanese waltzing mice employed were purchased on June 11, 1915. These were divided into five groups, one of which was placed in a contaminated cage, the other four in sterile cages. All food, water, sawdust, and dishes provided throughout the course of the experiment were previously sterilized. The five cages used were box cages about five inches deep and open at the top. These were placed on a table top and spaced several inches from one another. Less elaborate measures were taken with the common mice.

Procedure.	No.	Animal.	Result.
GROUP I. June 11. — Placed in contaminated cage.	5052	J. w. mouse.	Died June 21; typical lesions.
	5053	" " "	Died June 21; typical lesions.
	5054	" " "	Died June 22; typical lesions.
	5055	" " "	Killed June 26; typical lesions.
	5056	" " "	Dead July 1; typical lesions.
GROUP II. June 15. — Fed with diseased liver.	5042	J. w. mouse.	Dead June 25; typical lesions.
	5043	" " "	Dead June 29; typical lesions.
	5044	" " "	Dead June 30; typical lesions.
	5045	" " "	Dead July 1; typical lesions.
	5046	" " "	Killed July 2; typical lesions.
GROUP III. June 15. — Fed with the intestinal contents of diseased mice.	5047	J. w. mouse.	Dead June 26; typical lesions.
	5048	" " "	Dead June 28; typical lesions.
	5049	" " "	Dead June 28; typical lesions.
	5050	" " "	Dead June 29; typical lesions.
	5051	" " "	Dead July 2; typical lesions.
GROUP IV. June 26. — Inoculated subcutaneously with diseased liver.	5081	J. w. mouse.	Killed July 6; typical lesions.
	5082	" " "	Killed July 10; typical lesions.
	5083	" " "	Killed July 10; typical lesions.
	5084	" " "	Killed July 3; typical lesions.
	5085	" " "	Dead July 8; typical lesions.
GROUP V. June 11. — Furnished with sterilized food and cage (controls).	5091	J. w. mouse.	Dead July 12; typical lesions.
	5092	" " "	Killed July 10; typical lesions.
	5093	" " "	Killed July 10; typical lesions.
	5094	" " "	Dead July 12; typical lesions.
	5095	" " "	Dead July 6; typical lesions.

EXPERIMENT 1. — *Continued.*

Procedure.	No.	Animal.	Result.
GROUP VI. June 10, 22, 26, and 29. — Fed intestinal contents of diseased mice.	5028-A	Common mouse.	Killed July 7; liver normal.
	5028-B	" "	Killed July 7; liver normal.
	5028-C	" "	Killed July 7; liver normal.
	5028-D	" "	Killed July 7; liver normal.
	5028-E	" "	Killed July 7; liver normal.
GROUP VII. June 10, 22, 26, and 29. — Fed with diseased liver.	5029-A	Common mouse.	Killed July 7; liver normal.
	5029-B	" "	Killed July 7; liver normal.
	5029-C	" "	Killed July 7; liver normal.
	5029-D	" "	Killed July 7; liver normal.
	5029-E	" "	Killed July 7; liver normal.
GROUP VIII. June 26. — Inoculated subcutaneously with diseased liver.	5086-A	Common mouse.	July 9, diarrhea. Killed. Liver shows several minute spots. Organisms in lesion in skin.
	5086-B	" "	July 12. Killed. Normal, except subcutaneous nodule.
	5086-C	" "	July 12. Killed. Liver one minute whitish point. No bacilli here or beneath skin.
	5086-D	" "	July 17. Killed. Liver normal. Organisms, some in spore stage, beneath skin.
	5086-E	" "	Sept. 12. Killed. Normal.
GROUP IX. June 26. — Inoculated subcutaneously with diseased liver.	5087	Rabbit.	Sept. 12, normal. The implant had diminished in size and had disappeared.
GROUP X. June 26. — Injected intravenously with suspension of diseased liver.	5088	Rabbit.	July 12. Killed, although appearing healthy and vigorous. No lesions.
GROUP XI. June 26. — Inoculated subcutaneously with diseased liver.	5089	Guinea-pig.	July 27. Dead. Minute mass beneath skin, no bacteria found.
	5090	"	Sept. 30, normal. Nodule has disappeared.
GROUP XII. July 31. — Placed in infected cage.	5306	J. w. mouse.	Dead Aug. 10; typical lesions.
	5307-A	" " "	Dead Aug. 11; typical lesions.

From the above data it will be noted that the control Japanese waltzing mice eventually succumbed to the infection, although precautions were taken to avoid the interchange of any material between cages during the process of feeding. It appears probable from subsequent observations that at some time infectious material was flirted over into the control cage through the activities of the diseased mice. It is also to be questioned whether the disease occurring in the subcutaneously-inoculated mice was due to the inoculation, so that a repetition of this procedure was considered necessary. In some instances smears from the site of the subcutaneous inoculation showed slender bacilli and spores, the latter occurring in considerable numbers. The results obtained in Groups I., II., and III. indicate that cage contamination or the ingestion of the liver or intestinal contents of diseased mice serve equally well in producing infection, and that the average interval between exposure and death is approximately equal in the three groups. The average interval between purchase and death of the control Japanese waltzing mice is more than twice as long, indicating an infection derived from the experimentally-infected animals of Groups I., II., and III. From the infection of these control mice, it appears quite probable that only small, initial doses of the bacillus are required to produce the disease in susceptible animals, provided that conditions are furnished favorable for its multiplication.

Although infectious material was fed repeatedly to common mice no visible evidence of disease was produced. Of the common mice inoculated subcutaneously, two showed minute spots in the liver. These lesions consist of foci of leucocytic infiltration, but no bacilli are apparent in stained sections. Slender bacilli and spores were found in some instances, however, in smears from the site of the subcutaneous inoculation. From the absence of visible lesions, the possibility of intestinal infection in common mice was not considered at this time, and unfortunately no intestine was preserved.

Diseased liver implanted subcutaneously in guinea-pigs and in a rabbit, and inoculated intravenously in a rabbit, failed to produce the disease.

Intravenous injection of suspensions, prepared by grinding liver lesions in salt solution, into Japanese waltzing mice has furnished less uniform results. A Japanese waltzing mouse, which was injected with .2 cubic centimeter of such a suspension, died five days later, showed lesions similar to those of the natural disease, but rather more uniform in size and evenly distributed throughout the liver. There was also a minute spot on the wall of the right ventricle of the heart. In stained section the bacilli were found in great numbers in the liver parenchyma at the periphery of the lesions, but no spores were apparent. No bacilli were found in the intestinal epithelium. The injection of .1 cubic centimeter of suspension into the tail vein, and .3 cubic centimeter into the peritoneal cavity of a second Japanese waltzing mouse, resulted in lesions of the liver, which were apparent eight days later through the abdominal wall. The animal was killed twelve days after the injection, when it presented nine grayish lesions in the liver. By the examination of stained sections the lesions were found to consist of a centrally situated sequestrum of dead material surrounded by phagocytes, and a layer of newly formed connective tissue infiltrated with plasma and lymphoid cells. From such evidences of repair it is probable that the animal was well on the road to recovery when killed.

In each of the following two experiments a number of mice was injected intravenously with the infectious material, while others were inoculated intraperitoneally. The results obtained in the latter will be discussed separately in a subsequent paragraph.

EXPERIMENT 2.

One Japanese waltzing and three white mice were treated as follows:

No.	Animal.	Procedure.	Result.
5917 . .	J. w. mouse.	Oct. 24, 1916. Injected .1 cc. of suspension of liver lesions into tail vein.	Nov. 11. Dead. Three grayish lesions, and also a slight dimpling at intervals in the capsule of the liver. No bacilli. Condition indicates repair.
5918 . .	White mouse.	Oct. 24, 1916. Injected a minute amount of suspension of liver lesions into tail vein. Nov. 7, 1916. Injected .01 cc. of fluid from mixed culture containing *B. piliformis* subcutaneously. Dec. 9, 1916. Injected .15 cc. of a suspension of liver lesions into tail vein. Jan. 6, 1917. Placed in contaminated cage. Feb. 21, 1917. Killed.	All organs appear normal.
5919 . .	White mouse.	Oct. 24, 1916. Injected .2 cc. of a suspension of ground liver lesions, partly into the tail, the remainder at base of tail. Nov. 16, 1916. Killed.	Liver appears normal. A small area of consolidation in the lung.
5920 . .	White mouse.	Oct. 24, 1916. Injected intraperitoneally with .2 cc. of a suspension of ground liver lesions. Dec. 9, 1916. Killed. Tissues fixed for histological study.	All organs appear normal. Stained sections show extensive infection of the epithelium of large intestine and cecum. Both the bacillary and spore forms of *B. piliformis* are present in large numbers within cells.

EXPERIMENT 3.

No.	Animal.	Procedure.	Result.
5982 . .	J. w. mouse.	Dec. 7, 1916. Injected .33 cc. of a suspension of liver lesions ground in salt solution into tail vein. Dec. 14, 1916. Killed.	Six whitish lesions in liver ranging in size from minute spots to 2.5 mm. *B. piliformis* in smear of lesion.
5983 . .	J. w. mouse.	Dec. 7, 1916. Injected in similar manner. Dec. 14. On removing hair from belly a whitish spot apparent near border of liver. Now alive.	Infection followed by recovery. Immunity established, as shown later by repeated exposure to the infectious agent.
5984 . .	J. w. mouse.	Dec. 7, 1916. Injected in similar manner. Dec. 14. A whitish spot 1 mm. in diameter visible near border of liver. Now alive.	Infection followed by recovery. Subsequently immune, as shown by repeated attempts to produce the disease.

EXPERIMENT 3. — *Continued.*

No.	Animal.	Procedure.	Result.
5985 . .	J. w. mouse.	Dec. 7, 1916. Injected in similar manner. Dec. 19, 1916. Killed.	Five lesions present in the liver, the largest measuring 2.5 mm. in diameter.
5986 . .	d. Br. common mouse.	Dec. 7, 1916. Injected .33 cc. of a suspension of liver lesions ground in salt solution into tail vein. March 9, 1917. Killed.	All organs normal. No bacilli found in the intestinal epithelium, either in smear or stained section.
5987 . .	d. Br. common mouse.	Dec. 7, 1916. Similarly injected. March 9, 1917. Killed.	All organs normal. No bacilli found in the intestinal epithelium, either in smear or stained section.
5988 . .	d. Br. common mouse.	Dec. 7, 1916. Similarly injected with .66 cc. Dec. 14, 1916. Killed.	Liver presents three minute opacities. No bacilli found in stained smears of these.
5989 . .	d. Br. common mouse.	Dec. 7, 1916. Similarly injected with .66 cc.	Remained normal.
5990 . .	Common.	Dec. 7, 1916. Inoculated intraperitoneally with .66 cc. of suspension of ground liver lesions.	Jan. 3, 1917. Dead. Consolidation of right lung. Liver normal.
5991 . .	Common mouse.	Dec. 7, 1916. Inoculated intraperitoneally with .66 cc. of suspension of ground liver lesions.	Jan. 3, 1917. Dead. Lungs reddened. Liver normal.
5992 . .	Common mouse.	Dec. 7, 1916. Inoculated intraperitoneally with .66 cc. of suspension of ground liver lesions.	Jan. 9, 1917. Dead. Liver shows no characteristic lesions.
5993 . .	Common mouse.	Dec. 7, 1916. Inoculated intraperitoneally with .66 cc. of suspension of ground liver lesions. March 9, 1917. Killed.	*B. piliformis* not found in stained smears and sections of intestine.
5928 . .	J. w. mouse.	Dec. 7, 1916. Inoculated intraperitoneally with .66 cc. of suspension of liver lesions. Dec. 14, 1916. Killed.	Liver appears normal.
5929 . .	J. w. mouse.	Dec. 7, 1916. Inoculated intraperitoneally with .66 cc. of suspension of liver lesions.	Dec. 18, 1916. Dead. Liver appears normal.
5930 . .	J. w. mouse.	Dec. 7, 1916. Inoculated intraperitoneally with .66 cc. of suspension of liver lesions.	Dec. 18, 1916. Dead. Liver appears normal.
5931 . .	J. w. mouse.	Dec. 7, 1916. Inoculated intraperitoneally with .66 cc. of suspension of liver lesions.	Dec. 20, 1916. Dead. Liver presents no lesions.

It is evident from the above results that it is possible to produce in Japanese waltzing mice characteristic lesions of the liver by intravenous injections of the infectious material. The disease thus produced by moderate doses lacks the progressive character of the natural disease, although a large dose intravenously has caused extensive involvement of the liver and has shortened the course of the disease by one-half. In the latter case, the absence of intestinal infection indicates a failure to establish those conditions, which in the natural form of the disease make reinfection with constantly increasing dosage possible. The results obtained furnish additional evidence of the non-susceptibility of the common mouse to the disease presented by the Japanese waltzing mouse.

Intraperitoneal inoculation of Japanese waltzing mice with a suspension of diseased liver has failed to produce the disease. Four old mice bearing transplanted tumors were thus treated in Experiment 3. One killed seven days later, two dying eleven days, and the last dying thirteen days later showed no lesions of the liver. The presence of the bacilli in great numbers in the intestinal epithelium of common mouse No. 5920, in Experiment 2, shows that this organism may, occasionally at least, live parasitically in the latter variety without the production of visible disease. The intraperitoneal inoculation of four common mice in Experiment 3 failed to produce the disease, and *B. piliformis* was not found in stained sections of the intestinal tract of one killed three months later. This method of inoculation appears with this disease to be even less effective than intravenous injection with respect to causing infection.

Subcutaneous inoculation. — Since the results obtained by this method in Experiment 1 were indefinite, four Japanese waltzing mice were inoculated subcutaneously with liver lesions mixed with transplantable tumor. One of these died within a few days and showed no evidence of the disease. There was ulceration at the site of inoculation in another, and

this one died eighteen days after inoculation with numerous lesions in the liver and *B. piliformis* in the intestinal epithelium. The two remaining mice showed no ulceration and lived fifty-seven days, when they were killed. Several minute depressions were noted in the surface of the liver of each, but no definite evidence of disease. These results indicate that the Japanese waltzing mouse may be inoculated subcutaneously without fatal result and without generalization of the disease. In case ulceration takes place, as in the second mouse, intestinal infection may follow from licking the local lesion and the natural type of the disease may be produced. Both red forms and spores are found in considerable numbers at the site of the subcutaneous inoculation, so that it appears certain that there is multiplication of the organism locally. Common mice inoculated subcutaneously also showed local infection, which persisted for two and three weeks in certain of the animals examined.

Resistance of spores. — The long persistence of the spores of *B. piliformis* in contaminated material is shown by the following observation: In June, 1915, a cage in which diseased mice had been kept was wrapped in paper and set aside in the laboratory until the following year. In the meantime, the Japanese waltzing stock remained free from the disease. On June 6, 1916, two normal waltzing mice were placed in the contaminated cage. One was killed thirteen days later and showed several opacities, but no well-defined lesions in the liver. The second died thirty-seven days after exposure and presented numerous characteristic lesions in the liver. Since this bacillus has not yet been successfully cultivated, the resistance of the spores to heat has not been determined. Liver lesions from six mice were ground in salt solution and heated to 80° C. for one hour. Portions of this material were repeatedly either smeared on the fur or fed during the next twelve days to six mice. None of these subsequently showed any evidence of the disease. Since such results may very well be determined by dosage, as well as by the absence or presence of living spores, they must be considered as inconclusive in character.

Immunity. — The possibility of producing immunity through intravenous injections of small doses of the virus is shown in the results obtained with two mice (Nos. 5983 and 5984) in Experiment 3. These mice never appeared markedly ill, although lesions of the liver were seen through the body wall of each. One month after the injection the fur of each was smeared with liver lesions without fatal result. They were subsequently placed in a contaminated cage with normal Japanese waltzing mice. As the latter sickened, the food and other material became more and more foul from their abnormal intestinal discharges, notwithstanding which the two immune mice remained perfectly healthy. Normal mice subsequently placed in the cage with these have invariably succumbed to the disease after a brief period of incubation. In the meantime, each of the two immune females has raised a family of young. The first lot grew well, notwithstanding their foul surroundings, up to the time for weaning, when they all died. None of these showed liver lesions, and no characteristic spores or bacilli were found in smears of the intestinal epithelium. It is not improbable that other organisms present in such foul surroundings were sufficient to cause the death of such young animals. The second lot of three young were accordingly removed with the mother to a clean cage, where they grew unusually well. When thirty days old they and the two old immune females were exposed in a contaminated cage to test their resistance to the disease. All three young died within seven days without gross or microscopic evidence of infection. The two old immune females remained healthy.

It is evident, therefore, that a pronounced immunity may be produced by the intravenous injection of small doses of infectious material. It is probable that this may also be produced by subcutaneous or intraperitoneal inoculation, since neither has resulted fatally. No conclusive results have thus far been obtained concerning the question of the maternal transmission of acquired immunity to offspring.

There is no evidence that recovered mice serve as carriers, for, after the above immune females had been moved to

clean quarters, a young Japanese waltzing mouse placed with them failed to develop the disease.

Susceptibility. — Sex is not an important factor in the incidence of this disease of the Japanese waltzing mouse. Although it is apparent that practically all mice of this variety are susceptible, it is evident that old mice are somewhat more resistant, so that in them the course of the disease is more prolonged, while in young mice the disease is acute and quickly fatal.

The difference in the susceptibility of the Japanese waltzing and common varieties of mice is so extreme as to be of considerable interest. While great numbers of common mice were kept constantly on hand in the laboratories of the Cancer Commission, and while conditions were much more favorable for the transmission of parasitic organisms, lesions of the liver characteristic for this disease have been observed in only two instances. The failure of all attempts to produce the disease experimentally, furnishes further proof of the non-susceptibility of this variety. Of the one hundred and forty-four J. w. mice, which have shown this disease, the great majority have contracted it naturally or by mere exposure in contaminated cages. Practically the entire J. w. stock on hand and all newly purchased animals, no less than seventy-nine in all, succumbed while the disease was epidemic. The disease has appeared spontaneously in different types of hybrid mice, but exact data concerning their exposure to the disease are lacking. The number of cases of the disease thus far recorded is as follows:

	Cases.
Common mice (Br. Ag.)	2
Japanese waltzing mice	144
F_1 hybrids (one fatal, four killed)	5
F_2 "	11
F_3 "	1
F_4 "	1
$(F_1 \times$ J. w.$)^1$ back-cross hybrids	4
$(F_1 \times$ J. w.$)^2$ " " "	1
$(F_1 \times$ Br. Ag.$)^2$ " " "	1
$(F_1 \times F_2)^2$ hybrids	3
Total	173

It is evident that hybrids of the first filial generation (F_1) may become infected, for of six of these kept together in a cage, five developed the disease with characteristic liver lesions. The sixth died without showing visible lesions in the liver, but it was not determined whether there was intestinal infection. Of the five that showed liver lesions, one died of the disease and four were killed. From the above data it would appear that the F_1 hybrids are relatively susceptible to this disease, but it has not yet been determined how frequently recovery follows the infection. The histological examination of the livers of these F_1 hybrids showed both active and healed lesions in certain animals and advanced repair in one. In the second generation (F_2) hybrids, on the other hand, the disease is evidently not uniformly fatal. It has been repeatedly noted in lots of these that several would die of the disease, while the others in the same cage would remain apparently healthy. It appears important to test the susceptibility of various types of hybrids under conditions that invariably produce the disease in Japanese waltzing mice, and with this view hybrids are at the present time being reared for this purpose.

Whether *B. piliformis* is capable, when ingested, of infecting other species of animals has not been determined. It appears to have no pathogenic properties for guinea-pigs or rabbits when injected into their tissues. A young guinea-pig and a wood mouse (*Peryomyscus leucopus* Rafinesque) have been kept in a contaminated cage and fed with infectious material, but have remained apparently normal.

The disease under consideration thus presents certain unusual features. The bacillus (*B. piliformis*) with which it is associated is to be regarded as a peculiarly adapted cell parasite. This bacillus does not grow in masses within the epithelial cell, but the individual rods separate from one another in the cytoplasm, so that each comes to lie in a space by itself. In its relation to the cell it manifests a more perfect adaptation to cell parasitism than do most bacteria. The spore stage is commonly reached in the development of

this bacillus before the disintegration of the harboring cell is effected, and its spores are evidently shed in great numbers into the intestinal contents by the disintegration of the older epithelium at the surface of the mucosa. The rate of disintegration of the infected epithelium is probably not markedly greater than that which would occur normally, and does not exceed the rate of reparative growth. To what extent this organism may multiply in the intestinal contents cannot be readily determined. It appears probable that invasion is initiated by bacilli piercing the living cells in the course of their growth in empty goblet cells, or in crypts in the surface of the intestinal epithelium. It may then grow from the epithelium into the underlying tissues, penetrate the blood vessels, and be transported to the liver by way of the portal system. While it is possible that these organisms are sometimes carried to the blood by phagocytic cells, the fact that they grow into the sub-epithelial tissue and into the walls of the blood vessels is sufficient to account for their distribution in the liver. The dissemination of the infectious agent is probably favored by the close proximity of capillaries to the epithelium in the mouse's intestine. It appears that only the intestinal epithelium and liver parenchyma are adapted for their growth, for they have never been found in other organs of the body, even when the disease is produced by intravenous injection. Stained sections and smears from the mesocolic lymph nodes have failed to reveal any bacilli, so that there is no evidence that dissemination ever occurs through the lymphatics.

The transmission of this disease from animal to animal is effected by the contaminative method, that is, by contaminated food or surroundings, or by the direct ingestion of intestinal discharges. *B. piliformis*, after ingestion by the Japanese waltzing mouse, multiplies in the epithelium of the large intestine, the cecum, and the small intestine. Spores formed in the intestinal epithelium are shed in great numbers, and it is probable that these represent a resistant stage through which the organism is transmitted from host to host. The spores discharged in the feces of the infected animals

are undoubtedly ingested in large numbers by the latter, so that the dosage of the infectious agent is increased by the constant addition of ingested organisms to those already multiplying in the intestinal epithelium. That the severity of the disease depends to a large extent upon the continuous reingestion of the virus is shown by the prolongation of life, and the occasional recovery of infected animals that are isolated and kept in a clean environment, and also by the tendency of animals to recover from infection produced by intravenous injection.

Differences in the morphology of the bacilli are strongly suggestive of stages in a developmental cycle terminating in the formation of the spore. It is not improbable that the more uniformly stained organisms represent stages of a vegetative cycle, and that the banded forms in which the spores develop represent stages of a propagative cycle.

This organism appears to be only slightly pathogenic, possibly on account of the degree of its adaptation. Its profuse multiplication within the intestinal epithelium is associated with evidences of injury so insignificant that they are only found on careful microscopic examination. In fact the leucocytic infiltration may be no more marked than in intestines which are ordinarily regarded as normal. A great proportion of the infected epithelial cells are increased in size, but show no more than a slight departure from the normal. Thus it may in certain instances multiply extensively in the intestinal epithelium of the common mouse, without producing definite disease in this variety. The multiplication of the bacillus within the liver cells of the Japanese waltzing mouse excites a marked inflammatory reaction followed by necrosis, and a very fatal disease is produced. Its predilection for epithelium of a certain type is shown by its failure to multiply in other organs than the liver on intravenous injection. While the peculiar habits of the Japanese waltzing mouse undoubtedly favor the multiplication of this organism, it is evident that its pathogenicity for this variety of mouse is not dependent on this factor.

Failure to produce liver lesions in the common mouse by the repeated feeding of large amounts of infectious material or by intravenous injection, furnishes evidence of a marked difference in the capacity of resistance of the two varieties to the invasion of the tissues in this infection. While such differences in resistance are obviously inherited, it should be of interest to determine as much as possible concerning the nature of this inheritance.

By the intravenous injection of a large amount of the virus of this disease, a severe fatal infection may be produced, but the intravenous injection of moderate doses has produced a mild, self-limited infection which has been followed by complete recovery. A very pronounced immunity may thus be produced by intravenous injection of the liver lesions, and Japanese waltzing mice thus treated have survived under conditions which were invariably fatal to others of this variety. The infection of the intestinal epithelium, which is an important feature of the natural disease, has not been found in mice infected by intravenous inoculation. Neither intraperitoneal injection or subcutaneous inoculation has proved effective in producing the progressive type of the disease which follows the ingestion of infectious material.

Although attempts to isolate *B. piliformis* in pure culture have been thus far unsuccessful, the study of stained sections and smears of infected tissue has shown its stages of development under natural conditions. It is present in such numbers in the intestinal epithelium that much may be learned of its development by morphological study alone. Successive stages leading up to the formation of spores are readily recognized. Both the mode of dissemination of the organism within the host, and of its transmission to normal mice through spores, which are shed in great numbers from the intestinal mucosa, become apparent. Since this bacillus is apparently as perfectly adapted for intracellular parasitism as any of the Protozoa, it may be important to reconsider certain intracellular parasites heretofore regarded as belonging to the animal kingdom.

Concerning the origin of this disease of the Japanese waltzing mouse, all circumstantial evidence indicates the common

mouse as the source. While the disease is extremely rare in the latter, even where large numbers are kept in close quarters, it is possible that a mild infection of the intestinal tract may occur unrecognized in them. No evidence has been obtained, however, that it occurs generally, or even frequently, among either common tame or wild mice.

Since this organism is almost invariably fatal to the Japanese waltzing mouse, it is evident that it is not maintained in this variety under natural conditions. From the fact that in the Japanese waltzing mouse we have an artificial variety, in all probability, obtained by the inbreeding of mutants, it appears quite possible that we are dealing with the first experience of this variety with the infection in question.

REFERENCES.

1. McJunkin, F. A. A polychrome stain with advantages over the Giemsa. Jour. Am. Med. Assoc., 1915, lxv, 2164.

2. Flexner, S. A study of the Bacillus (Leptothrix?) pyogenes filiformis (Nov. spec.) and of its pathogenic action. Jour. of Exper. Med., 1896, i, 211.

DESCRIPTION OF PLATES IX.–XI.

(Photographs, Plate IX., by L. M. Leavitt. Drawings, Plate XI., by E. Piotti.)

PLATE IX.

FIG. 1. — The liver of an infected Japanese waltzing mouse, showing numerous lesions, mouse No. 6045. x 2.

FIG. 2. — A small lesion of the liver consisting of polymorphonuclear leucocytes and fibrinoid material in place of liver parenchyma. A portal vein may be seen extending radially into the lesion from below and to the right. Stained section x 100. J. w. mouse No. 5084.

FIG. 3. — A liver lesion in which the process of repair is far advanced. The portal vein extends into the lesion from below and to the right. In the same liver were other lesions showing less evidence of repair and others in the acute stage with active invasion of the bacilli. Stained section x 100. F_1 mouse No. 5793.

FIG. 4. — Large banded forms of *B. piliformis* in the liver cells at the periphery of a lesion. Stained section x 1,400. F_1 mouse No. 5790.

FIG. 5. — Immature spores and bacillary forms of *B. piliformis* in the epithelium of the large intestine of the Japanese waltzing mouse. Stained section x 1,400. Mouse No. 5084.

FIG. 6. — Mixed culture derived from an infected liver. Bacilli having the morphology of *B. piliformis* together with streptococci. Two immature spores in the upper half of figure. Smear x 1,400.

FIG. 7. — Epithelial cells from large intestine showing above the long slender forms and below the shorter thicker form of *B. piliformis*. Smear — spore stain x 1,400. J. w. mouse No. 6013.

FIG. 8. — A pale, degenerated epithelial cell, in which are a number of immature spores. Smear — spore stain x 1,400. J. w. mouse No. 6013.

FIG. 9 — Intestinal epithelium. The upper cell contains a group of developing spores and a few slender bacillary forms. Slender rods are present in the lower of the two cells. Smear — spore stain x 1,400. J. w. mouse No. 6013.

PLATE X.

FIGS. 10 to 16 show groups of *B. piliformis* found in a smear of the bile from a case of severe infection. Each group is presumably derived from a single cell, in fact the remains of the cell in which they have grown is apparent in several instances. The groups are arranged to show successive stages of development, terminating in the formation of spores. The slender, tapering organisms seen in Fig. 10 give rise to shorter, thicker forms, which are distinctly banded. The substance forming the interior of the spore stains intensely until the latter becomes mature, when it stains with difficulty, probably owing to the formation of a resistant membrane. x 1,490.

FIG. 17. — Various forms of *B. piliformis* stained with McJunkin's stain — a, short rods; b, large, banded forms; c, stages in the development of spores; d, looped form; e, U-shaped organism with central swelling. x 1,400.

FIG. 18. — *B. piliformis* — Loeffler's alkaline methylene blue stain — a, long forms, with slight evidence of banding; b, short rods, probably derived from the division of longer forms; c, larger, thicker rods; d, a twisted form, indicating the non-rigid character of this organism; e, over-stained banded rods; f, long forms, with terminal and central swellings. x 1,400.

FIG. 19. — Spores showing variation in the appearance of the attached rod. x 1,400.

FIG. 20. — A portion of the periphery of a liver lesion in stained section showing the sharp line of demarcation marked by a deposit of fibrin and the bacilli distributed in the surrounding liver cells. Polymorphonuclear leucocytes are apparent within the lesion in the left-hand portion of the drawing. x 1,400.

FIG. 21. — An epithelial cell from the large intestine containing both immature spores and slender bacilli. Smear — spore stain x 1,400.

FIG. 22. — Bacilli in a cell lying against the wall of a capillary extending just beneath the intestinal epithelium. To the right is a group of spores, also situated beneath the epithelium. Stained section x 1,400.

FIG. 23. — Large, banded forms of *B. piliformis*, one with a centrally situated swelling situated in a liver cell. Other more slender organisms are also apparent. Stained section x 1,400.

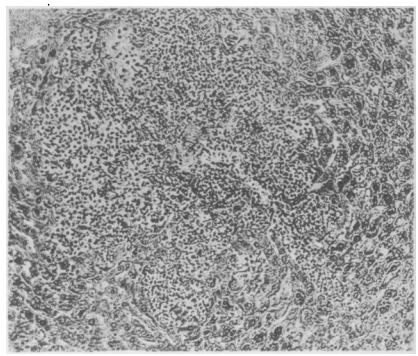
PLATE XI.

FIG. 24. — Superficial portion of the mucosa of the large intestine of a severely infected mouse. The bacilli appear like dots when seen from the end. They are not arranged in masses, but each lies separate in the cytoplasm. Variation in the thickness and in the staining of the bacilli is apparent. Spores are numerous in the surface epithelium, and are also present near the lumina of the glands. Stained section x 1,400. J. w. mouse No. 6013.

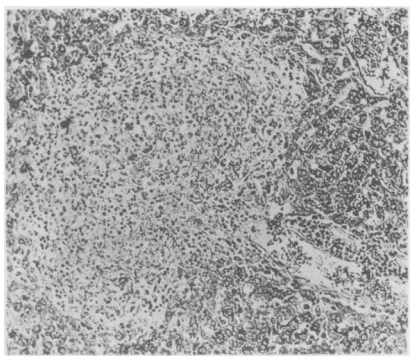
FIG. 25. — Long, banded bacilli in a lymphatic lying against the epithelium, seen to the left and in close proximity to a blood vessel. Stained section x 1,400. J. w. mouse No. 6013.

FIG. 26. — Early invasion of the liver parenchyma in close proximity to a portal vein apparent to the right of the figure. Only the rod forms of *B. piliformis* are present. Stained section x 1,400.

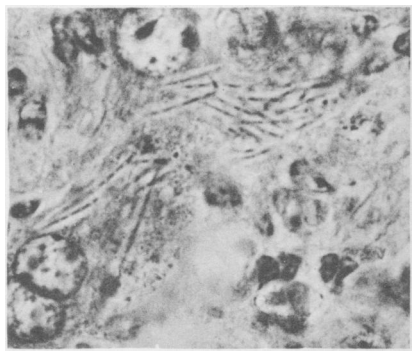
1



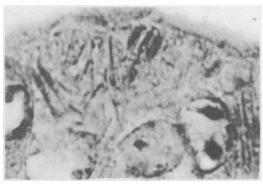
2



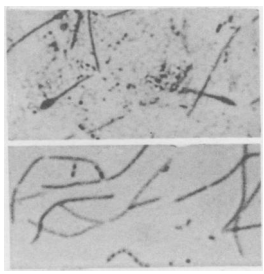
3



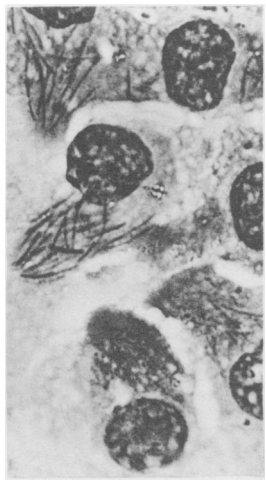
4



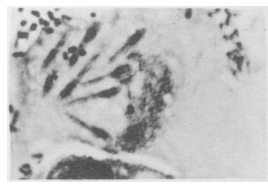
5



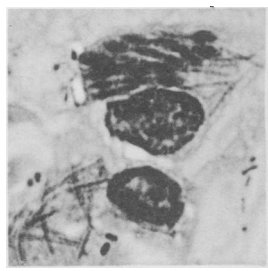
6



7



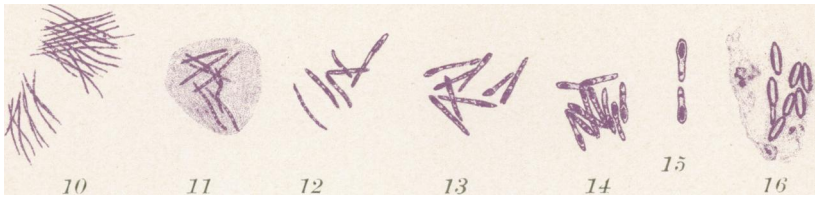
8



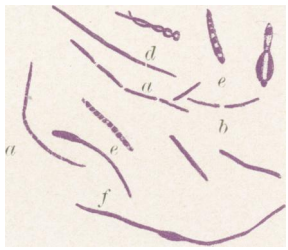
9

Tyzzer.

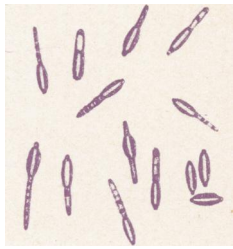
Japanese Waltzing Mice.



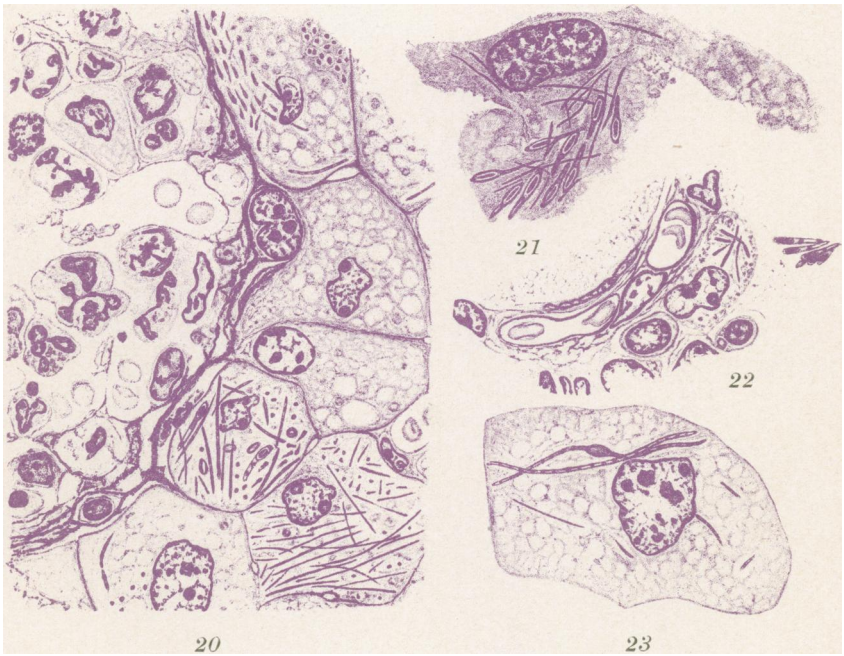
17

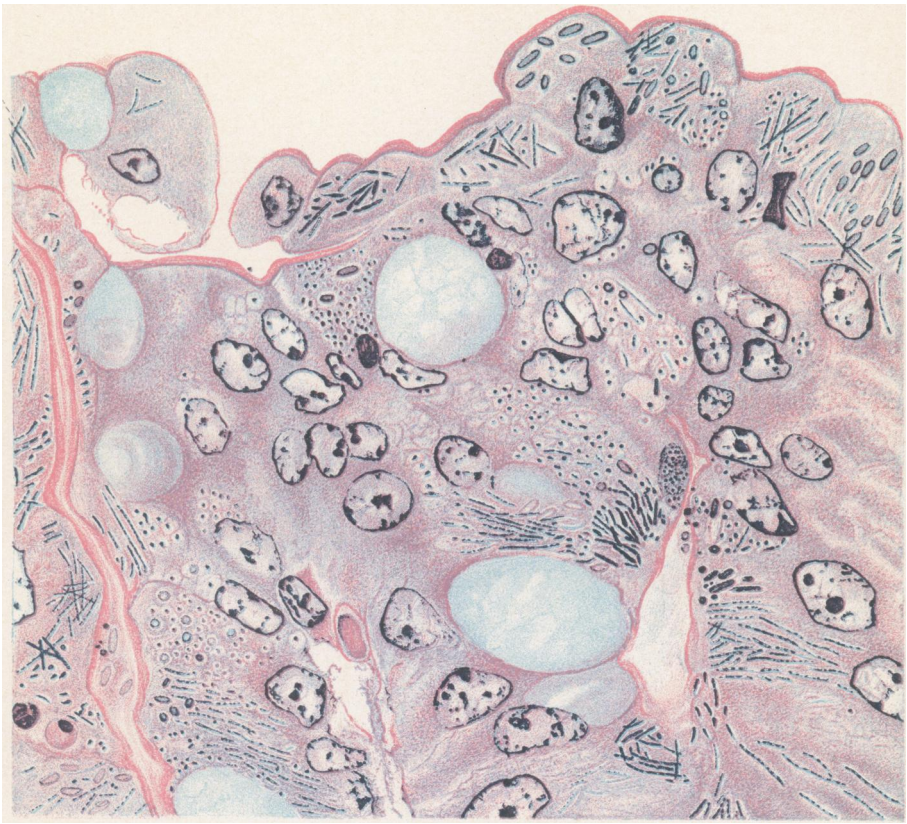


18



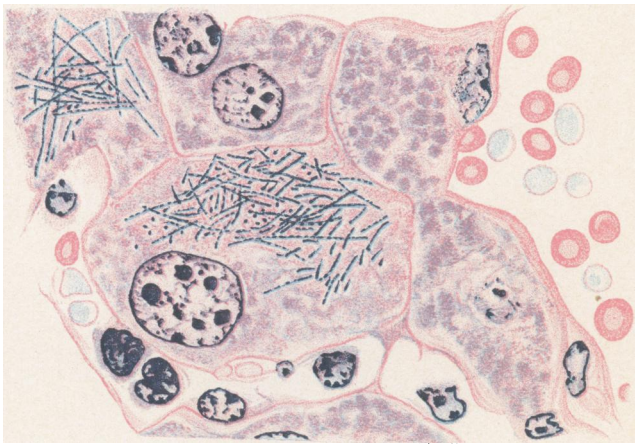
19





24

25



26

Tyzzer. Japanese Waltzing Mice.