OBSERVATIONS ON TYZZER'S DISEASE IN MICE *

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A spontaneous disease of Japanese waltzing mice characterized by large focal inflammatory lesions of the liver was noted and described by Tyzzer [1] in 1917. His excellent photomicrographs illustrate the long, banded, bacilliform organisms found in affected cells of the liver and intestinal mucosa. Although the organism associated with the disease could not be cultivated, it was regarded as the etiological agent and called *Bacillus piliformis,* solely on morphological grounds. Experimental transmission of the disease to normal waltzing mice was accomplished with some regularity by contact with diseased animals or by the ingestion or intravenous injection of very large doses of infected tissues. However, the appearance of the disease in various control groups indicated its general presence throughout these transmission experiments. Ordinary laboratory animals were not susceptible, but the disease was later found by Tyzzer [2] in certain inbred strains of mice being used for cancer studies.

A disease apparently identical with that described by Tyzzer was encountered in a British strain of Swiss mice during the functional activities of the First Medical General Laboratory, U. S. Army, in England. The present report reviews briefly the natural infection and the characteristics of the associated organism; in addition, it describes for the first time the regular and consistent experimental transmission of this disease in mice and other laboratory animals, and the cultivation of the organism in tissue cultures.

MATERIALS AND METHODS

Laboratory Animals. The laboratory animals employed in these studies, including the Swiss mice in which the disease was enzootic, were obtained from the British Agricultural Experiment Station at Compton, England. The farm strain of mice was an inbred stock which had originated from the cross breeding of albino with wild mice.

Culture Media. The basal medium employed in the present work was standard beef heart infusion broth prepared from the dehydrated powder † or from fresh material.[3] This broth base contained 0.8 per

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cent NaCl and 1 per cent of neopeptone or proteose peptone. Various combinations of blood, serum, glucose, ascorbic acid, and thioglycollate were added to this medium for enrichment. The Löffler's, Löwenstein-Jensen's, and Sabouraud's media were the standard preparations used in diagnostic bacteriology.[3]

Cultural Methods. Cultures were incubated at 37.5°C. except in a few instances when parallel cultures were carried at room temperature (20 to 23°C.). An atmosphere of 10 to 12 per cent CO_2 was obtained by the candle jar method. A McIntosh-Fildes' jar was used to obtain anaerobiosis which was checked by a methylene blue-alkaline glucose control tube.

Tissue Cultures. Agar slope tissue cultures were prepared according to the method described by Zinsser, Wei, and Fitzpatrick.[4] The minced tissues of either 10-day-old chick embryos or of 15 to 20-day-old mouse embryos served as a source of cells. A 10 per cent suspension of infected mouse brain was added to the embryonic tissue and the mixture was spread on serum agar slopes; the tubes were stoppered with rubber plugs and incubated aerobically at 37°C. for 5 to 10 days.

Histological Methods. Tissue specimens for microscopical examination were fixed in Zenker's formalin solution; paraffin sections were stained with hematoxylin and eosin, by Giemsa's method, or by Weigert's modification of Gram's stain.

Experimental Findings

The Natural Infection

Outbreaks of the natural infection occurred in the mouse colony in the late winter and early spring of 2 successive years. The epizootic was associated in each instance with the overcrowding of breeding stocks. Mice which succumbed to the disease showed only vague, indefinite signs of illness consisting of a ruffled coat, an emaciated appearance, and occasionally a mild diarrhea. The autopsy findings, which were essentially the same as those described by Tyzzer, revealed a liver mottled by large (1 to 2 mm.), hard, yellow, inflammatory lesions with punctate necrotic centers. These were visible through the abdominal wall after the skin had been reflected. No other macroscopic lesions were observed consistently. Histological examinations of the hepatic lesions showed that the characteristic structure consisted of a central area of necrosis frequently surrounded by a zone of infiltrating polymorphonuclear cells with a peripheral area in which the parenchymatous cells had undergone some necrobiotic changes (Fig. 1). In this marginal zone, hepatic cells were seen frequently with their cytoplasm

filled with the organisms associated with the disease. These organisms were regularly demonstrated microscopically in stained impression smears of infected tissue and in smears of suspensions of ground tissue. They appeared as gram-negative, long, thin, nonbranching, multiple, banded rods which were often fusiform (Fig. 4). Although pleomorphic bulbous structures of the type described by Tyzzer were seen occasionally, we were not convinced that they warranted a classification of the organism as a member of the spore-forming group. Dark-field examinations of infected tissue suspensions failed to demonstrate motility.

Experimental Transmission of the Disease in Mice

Attempts were made to transmit the spontaneous disease of Swiss mice to apparently healthy mice of the same stock with suspensions of affected liver tissue. In one series of experiments, groups of 10 mice were either forcibly fed or inoculated by the intraperitoneal, intravenous, or subcutaneous route with such suspensions. No significant increase in incidence of hepatic disease was found in the treated groups during a period of 40 days' observation, when compared with the untreated control group. In contrast, the intracerebral inoculation of mice with suspensions of affected liver tissue resulted in each of three instances in the production of an acute encephalitis in the treated animals. The experimental disease was transmissible by intracerebral injection of 10 per cent suspensions of brain tissue from infected mice. One strain, called ML-7, was maintained in this manner through 100 serial transfers; the work with this strain provided most of the material for the present study. This method of producing an experimental encephalitis is important for the further investigation of the disease since transmission by other methods has not been satisfactorily consistent, and the etiological agent does not grow on ordinary culture media.

The Experimental Encephalitis in Mice

Swiss mice inoculated intracerebrally with suspensions of infected tissue usually died with signs of disease of the central nervous system between the third and the eighth day after injection. Affected animals developed increased irritability and paralysis of one or more limbs followed by convulsions and death, all in the course of a few hours. Neither the brain nor the viscera of mice dying with encephalitis showed macroscopic lesions. Histological examination of affected brains revealed areas of complete liquefaction necrosis which were sometimes surrounded by a moderate infiltration of polymorphonuclear cells (Fig. 2). The characteristic banded organisms were frequently

seen at the periphery of the necrotic foci; these were generally in an intracellular position (Fig. 5).

The Susceptibility of Other Laboratory Animals

Farm mice, white rats, rabbits, and hamsters were tested for their susceptibility to the experimental encephalitis. Farm mice were as susceptible to this infection by the intracerebral route as were the Swiss mice from which the agent originally had been recovered. Rats, rabbits, and hamsters also developed encephalitis when injected intracerebrally with 10 per cent suspensions of brain tissue infected with the agent of mouse liver disease. Microscopical examination of the brains of these animals revealed the presence of lesions similar in all respects to those found in experimentally infected mice. Figure 3 illustrates a lesion in the brain of an infected hamster.

Cultivation of the Piliformis Organism

The cultivation of the piliformis organism on cell-free media has been unsuccessful in our hands. In addition to the ordinary bacteriological culture media, a number of enriched media were employed under various environmental conditions. Certain of these media were of the type ordinarily employed for the cultivation of pleuropneumonia organisms. Preparations of such inoculated fluid and semisolid media were stained by Giemsa's as well as Gram's method for microscopical examination. A summary of the work is presented in Table I.

Some success was achieved, however, when the cultivation of this agent was attempted in serum agar slope, tissue cultures. Tissue cultures were examined microscopically for evidence of growth of the typical organisms and were tested for the presence of bacterial contaminants by the routine inoculation of blood agar plates. In addition, the pathogenicity of these tissue cultures was determined by the intracerebral inoculation of mice. Only slightly encouraging results were obtained when the cultivation of the piliformis organism was attempted on tissues from minced chick embryos. In three separate series of experiments, a moderate number of morphologically typical organisms were present in the primary culture and these proved to be virulent for mice; subcultures, however, contained only a few organisms and were completely avirulent.

In contrast, the use of embryonic mouse tissue in the agar slope cultures met with more success. Cultivation in this medium resulted in an abundant growth of the piliformis organisms which appeared typical of those seen in stained smears of material from the natural or experi-

mental lesions in mice. Materials from the original and first subculture were virulent for mice, and microscopical examination of the brains of the animals which succumbed showed that they contained piliformis organisms. Although the second, third, and fourth subcultures were rich in organisms, they failed to induce obvious disease in mice. Identical results were obtained on another occasion in a similar series of cultures.

TABLE I

Culture Media Used in Attempted Cultivation of the Piliformis Organism

Base	Medium	Enrichments	Ph	Atmospheric environment	Period observed (*days*)
Beef heart infusion	2% agar	8% sheep blood	7.2–7.6	Air	10
Beef heart infusion	2% agar	8% sheep blood	7.2–7.6	10% CO_2	10
Beef heart infusion	2% agar	8% sheep blood	7.2–7.6	Anaerobic	10
Beef heart infusion	2% broth	0.05% thioglycollate	7.2–7.6	Air	10
Beef heart infusion	2% broth	0.05% thioglycollate + 10% horse serum	7.2–7.6	Air	10
Beef heart infusion	Broth	0.2% dextrose + 20% horse serum + 0.05% ascorbic acid	7.2–7.6	Air	10
Beef heart infusion	Broth	0.2% dextrose + 20% horse serum + 0.5% ascorbic acid	7.2–7.6	Anaerobic	10
Beef heart infusion	Broth	5% rabbit blood	7.8–8.0	Air	14
Beef heart infusion	2% agar	5% rabbit blood	7.8–8.0	Air (moist chamber)	14
Beef heart infusion	Broth	30% horse serum	7.8–8.0	Air	14*
Beef heart infusion	2% agar	30% horse serum	7.8–8.0	Air (moist chamber)	14
Beef heart infusion	Broth	20% rabbit serum	7.8–8.0	Air	14
Beef heart infusion	1.5% agar	25% rabbit serum	7.8–8.0	Air (moist chamber)	14*
Löwenstein-Jensen		None		Air-sealed caps	21
Löwenstein-Jensen		0.05% thioglycollate		Air-sealed caps	21
Sabouraud's*		2% dextrose			10

* At least 2 or more blind passages were made in this medium.

All media were inoculated with 10% suspensions of mouse brain which were known to contain the agent since they subsequently killed normal mice when injected intracerebrally.

In every case the organisms grown in the tissue cultures were not ordinary bacterial contaminants, since they failed to multiply on blood agar plates. In summary, the piliformis agent grew abundantly in embryonic mouse tissues cultures but lost its virulence for mice after a few such serial passages.

Effect of Storage on the Agent of Tyzzer's Disease

The infectivity of mouse brain or liver tissue, either in the form of 10 per cent suspensions or as blocks of organs, was destroyed by storage in the frozen state for longer than 2 weeks. However, the agent

was regularly recoverable from infected tissues stored at −20° or −70°C. for only a few days.

Discussion

Overcrowding undoubtedly provided one of the important factors in the explosive epizootics of liver disease which occurred among our mice. On each occasion of an outbreak, it was found that the mice, after receipt from the breeder, had been kept for some time under unsatisfactory conditions. The disease returned to a sporadic state when animals in subsequent shipments were provided with adequate quarters. The marginal war diet given mice may have contributed to their susceptibility, but it was probably not a factor in the sharp outbreaks since essentially the same foodstuffs were fed by both the breeder and us. It would appear that the disease was enzootic in the British stock mice and that it flared up when conditions were adverse, a common epidemiological observation. Some evidence for its frequent presence was the finding on a number of occasions of structures which appeared typical of the piliformis organism in stained smears of spleens of apparently normal mice of the same stock. Tyzzer likewise regarded the disease as one which occurred enzootically.

The part the piliformis organism plays in the etiology of mouse liver disease has not been clearly established. In our experience, as in Tyzzer's, this organism was always present in material infectious for mice. Indeed, the incubation period of the experimental disease was usually inversely proportional to the number of organisms seen in infected mouse tissue employed as inoculum. However, in certain instances obvious illness was not induced in mice by inocula rich in the organisms; notably, in those experiments with piliformis grown for several passages on tissue cultures.

These observations may indicate that the piliformis organism is the sole etiological agent of the fatal encephalitis in mice but that it is a fastidious organism which rapidly loses its virulence when grown in tissue culture. On the other hand, they may indicate that piliformis and an associated agent are responsible for the mouse disease and that piliformis grows in tissue culture but the concomitant agent does not. Serological studies, which unfortunately we were unable to complete, should contribute to an understanding of this problem. Van Rooyen [5] has commented on the morphological similarity of *Bacillus piliformis* and *Streptobacillus moniliformis*, but further analogies between these organisms are lacking. Neither is there weighty evidence which suggests that the agent of Tyzzer's disease is a member of the pleuropneumonia group of organisms.[6]

SUMMARY

Tyzzer's disease (epizootic hepatitis) of mice can be transmitted experimentally to mice, rats, hamsters, and rabbits by the intracerebral inoculation of infectious material. The experimental cerebral lesions, like those occurring in the livers of naturally infected mice, consist of focal areas of liquefaction necrosis surrounded by an acute inflammatory infiltration and contain many of the bacteria-like piliformis organisms. The piliformis organism is consistently associated with the natural and experimental disease in animals, and can be grown in tissue culture, but has not yet been cultivated in media devoid of living cells.

The photomicrographs were prepared under the direction of Mr. Roy M. Reeve at the Army Institute of Pathology, Army Medical Museum, Washington 25, D.C., where the negatives are on file.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 106

FIG. 1. Photomicrograph illustrating a typical lesion in the liver of a naturally infected mouse. There is an area of liquefaction necrosis surrounded by a zone of infiltrating cells. Hematoxylin and eosin stain. × 100. (Neg. no. 93334.)

FIG. 2. Photomicrograph illustrating acute necrosis in the brain of a mouse with experimental encephalitis. Hematoxylin and eosin stain. × 100. (Neg. no. 93157.)

FIG. 3. Photomicrograph showing the edge of a zone of necrosis in the brain of a hamster injected intracerebrally. Polymorphonuclear cells are found in the disorganized adjacent area. Hematoxylin and eosin stain. × 600. (Neg. no 93339.)

FIG. 4. Photomicrograph of piliformis organisms found in the hepatic lesion of a mouse with the natural disease. Of note is the characteristic banded appearance of the organisms. Gram's stain (Weigert's method). × 1350. (Neg no. 93155.)

FIG. 5. Photomicrograph of a section of the brain of a mouse dying from experimental encephalitis. Clusters of the organisms are seen in the remnants of cells and lying loose in the tissue. Gram's stain. × 1360. (Neg. no. 93340.)