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Host-Parasite Relations in Initiation of Infection - I. Occurrence of Listeriosis in Arctic Mammals, with a Note on its Possible Pathogenesis.

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ABSTRACT

A lemming colony maintained at Fort Churchill was examined for the possibility of the presence of *L. monocytogenes* in latent form, but the organism was not isolated. Following transfer of the lemmings from Churchill to Kingston, *L. monocytogens* was isolated. In speculation on the pathogenesis of Listerosis, the author suggests that an upset carbohydrate metabolism may be involved in the trigger mechanism.

The isolation of *L. monocytogenes* from a seemingly ill Arctic fox caught at Fort Churchill is reported for the first time.

INTRODUCTION

Owing to the occurrence of several cases of illness of uncertain etiology in personnel at Fort Churchill, one of whom was an animal caretaker, a request was made for an investigation into the possibility that *L. monocytogenes* might be present in a latent form in the lemming colony maintained there.

Listeriosis is the result of infection with *L. monocytogenes*. In man the disease is usually associated with meningitis or meningo-encephalitis. Listeriosis in man has been linked with Infectious Mononucleosis owing to the fact that *L. monocytogenes* on several occasions has been isolated from the latter condition, and that a mononucleosis is a manifestation of both diseases. The relation is not clear.

L. monocytogenes was first isolated by Murray, Webb and Swan in England in 1926 during an epizootic among Laboratory rabbits. In rodents it generally takes the form of a systemic infection, with or without meningeal complications and associated with a monocytosis. Necrosis of the liver is a constant finding. In the ruminant the infection occurs as an encephalitis.

L. monocytogenes is a gram-positive short rod, motile at room temperature, non-motile when grown at 37°C.

LITERATURE SURVEY

Isolation of *L. monocytogenes* has previously been isolated on two occasions from lemmings originating in the far north. Plummer and Byrne isolated the organism from a group of 24 lemmings, captured at Chesterfield and Morse Island in the fall of 1949 and shipped to Ottawa, where they died of Listeriosis (1). These authors speculated that lemmings might be carriers of *L. monocytogenes*. Levy (2) suspected the vole. The second isolation, made by Barrales (3), was from 40 lemmings shipped from DRNL at Fort Churchill to the School of Hygiene, Toronto, in the fall of 1952. A total of 35 lemmings died in 5 weeks.

It may thus be assumed that the organism exists in the north in a latent form in this species, and that external factors play an essential part in precipitating frank infection.

Further evidence is added by the isolation of *L. monocytogenes* from the Arctic fox, reported for the first time in this paper. The Arctic fox and owl are predators on the lemming (4), and the population cycles of these three species coincide closely (5). Many other predators of the lemming exist (6), such as the ermine, wolf, bear, raven, falcon, jaeger, and gull. Even the caribou is reported to eat lemming occa-

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sionally, probably by accident (7). Man may become a predator, sometimes in case of emergency and sometimes from choice (6).

It is possible that unfavourable conditions in animal populations at times pave the way for frank infection in individuals and result in epizootics. If this is so, listeriosis may well be an additional cause of the population fluctuation of the lemming (8) and of many other wild animal species. Following the periodic sudden declines in the lemming population (lemming crashes) their predators, if infected, might in their search for other food, conceivably carry the infection south, and thus indirectly be the cause of the generally more sporadic cases in man and of the more definite epizootics occurring in the domestic animals.

A large amount of undiagnosed disease exists in man in the Arctic (9), particularly among the Eskimos and Indians, which in part may stem from the animal population. This organism might therefore present a public health problem in the north, not only for the native population but for the increasing numbers of military and civilian personnel.

Experimental infection with *L. monocytogenes* by various routes, not including the respiratory, has been reported in several of the domestic animals, in the guinea pig and in mice (10).

This paper reports the examination of lemmings, voles, and foxes for the presence of *L. monocytogenes* and presents speculations concerning the pathogenesis of Listeriosis.

SURVEY OF THE LEMMING COLONY AT FORT CHURCHILL

The investigation, undertaken in the fall of 1953, consisted of a bacteriological, immunological, and pathological survey of 38 lemmings, 26 of which were born in captivity, 11 were trapped during the preceding year, and one was trapped during the investigation.

In addition, 4 voles were trapped and included in the survey.

Also, the findings in 3 Arctic foxes, together with a report on 12 lemmings and 4 voles which were shipped to Kingston, are included.

The animals in the colony, which consisted of a mixture of brown (*Dicrostonyx groenlandicus richardsoni*) and grey (*Dicrostonyx groenlandicus groenlandicus*) lemmings, were found to be in good health as evidenced by general appearance and good appetite coupled with the fact that a successful breeding program was carried out. No unexplained deaths were said to have occurred for some time previous to this investigation.

Although an attempt was made in 1950 to determine the cause of the deaths which had occurred in the colony, it was found that of those animals whose deaths were due to some identifiable cause, the largest single group exhibited symptoms of a condition diagnosed as diabetes mellitus (11).

Methods

The animals were killed by ether administration. During the stage of anaesthesia preceding death, a blood sample was obtained for the serological study by incising the axillary regions, severing the axillary artery. The pocket produced in this fashion between the front leg and the chest wall was employed as a sterile receptacle from which the blood was easily collected by means of a Pasteur pipette. At the same time, blood smears were prepared for differential counts.

At autopsy, tissues from the brain and liver and at times from the heart, lungs or spleen were removed and placed in individual tubes of tryptose broth, which were immediately placed in the refrigerator. At the same time, blocks of tissue from the same organs were placed in 10% Formalin for the histo-pathological study. From these, paraffin sections were later made and stained with haemotoxylin and eosin.

The tubes of tryptose broth were brought to the Defence Research King-

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ston Laboratory. During transit they were packed in a wooden box with a compartment containing dry ice. The transfer took 22 hours. On arrival at the Laboratory at Kingston the tubes were again placed in the refrigerator.

As time permitted, the tryptose broth tubes containing the tissues were emptied into tissue grinders and macerated. The macerated tissue suspensions were poured into Erlenmeyer flasks containing glass beads and about 15 ml. of tryptose broth. They were agitated in a rotary shaker for 20 min. and replaced in the refrigerator (12). Periodically, a portion of the macerated tissue suspensions were plated out on tryptose agar and incubated at 37°C for 24 hrs. On a few of the plates contaminants developed, consisting mostly of staphylococci and pseudomonas.

No animal from the lemming colony, killed at Fort Churchill, has yielded *L. monocytogenes* to date.

Histo-pathology

Four animals showed chronic hepatitis as evidenced by an increase in the number of round cells in the portal areas of the liver. Two of these four animals in addition showed an increased number of cells in the subarachnoid space of the brain, suggesting chronic meningitis.

One animal showed a chronic meningitis without hepatitis.

One animal showed lesions in the liver consisting of necrosis and focal accumulations of acute inflammatory cells scattered indiscriminately in different parts of the liver lobule.

In one, a myocardial scar was present.

One showed focal accumulations of chronic inflammatory cells in the brain tissue indicative of encephalitis.

In three animals, acidophilic intranuclear inclusion bodies were noted in the liver.

It is not implied that these findings are characteristic lesions of Listeriosis. They are, however, in some cases suggestive.

The blood films revealed no abnormal findings.

Serological Survey

Twenty of the sera from 34 animals tested showed agglutinating titres of 1:20 to 1:80 against a somatic antigen prepared according to Paterson (13). In addition, the one lemming trapped immediately before being killed showed a definite titre of 1:160, with partial agglutination up to and including 1:640.

Of these 21 positive sera, eight were from the ten animals exhibiting various lesions. Six of these eight (75%) gave positive titres of 1:40 to 1:160 (4 — 1:40; 1 — 1:80; 1 — 1:160) compared with 15 positive sera (58%) from 26 animals without lesions. The titres of the latter group ranged from 1:20 to 1:80 (7 — 1:20; 7 — 1:40; 1 — 1:80).

In addition to the sera from the animals enumerated above, sera from three young female dogs belonging to personnel at Fort Churchill were tested. They showed agglutinating titres of 1:20 to 1:80.

Also, of four serum samples from Eskimos, one agglutinated to a titre of 1:40 one to 1:20, and two were negative. Of four sera from Whites, one agglutinated to a titre of 1:20, 3 were negative.

As the agglutinating titre normally fades rapidly after recovery, it cannot serve reliably for late diagnosis, but a high titre (over 200), or better a rising titre, is significant (14).

In view of this, an interpretation of these serological findings should perhaps not be attempted. However, combined with the histopathological findings, they might suggest the presence of infection, past or latent, in the colony.

THE ISOLATION OF L. MONOCYTOGENES FROM AN ARCTIC FOX

An arctic fox (Alopex Lagopus) was captured at Fort Churchill in September, 1954. It behaved as if it were mentally deranged. Because the presence of rabies was suspected in the area, it was kept under observation until the possibility of rabies was eliminated, when it was

killed by ether administration. Just prior to death a blood sample was obtained by heart puncture.

Bacteriology

Growth was obtained on the plates inoculated with the material from the brain and from the heart suspensions. The colonies were small, less than 1 mm. in diameter in size, smooth, glistening and translucent. They appeared greenish with a finely textured surface, when observed under a dissecting microscope with transmitted light.

The gram stain revealed gram-positive rods, somewhat diphtheroid in appearance. Stab inoculation into semi-solid medium (8% gelatin) revealed growth along the entire stab and in the immediate surroundings, with a few puffball extensions into the medium (15).

When grown at room temperature, the organism was found to be actively motile, with a tumbling type of motility. When grown at 37° it was non-motile. In broth, a slight and uniform turbidity developed along with a heavy sediment.

In 48 hours, acid without gas was formed from glucose, lactose, sucrose, maltose, salicin, xylose, levulose, rhamnose and galactose; more slowly and incompletely from arabinose, sorbitol and esculin. Dulcitol, inulin and inositol were not fermented. Litmus milk was little changed; there was no coagulation. Hydrogen sulphide was not produced, indol was not formed, and nitrates were not reduced. The Voges-Proskauer and Methyl-Red tests were negative. The catalase test was positive.

When 1.5 ml of pure culture of this organism was injected intravenously into a rabbit, death occurred in 24 hours. The organism was re-isolated from the brain and heart of this rabbit, and a culture of each similarly injected into two rabbits. (Nos. 2 and 3); these last two rabbits both died in 24 hours.

Pathology

Histo-pathological examination of the tissues from rabbit No. 2 showed a subacute meningitis, and from rabbit No. 3 a sub-acute meningitis as well as focal necrosis of the liver. When 0.5 ml of the same culture was injected intravenously into a rabbit, death did not occur. Illness was evident for a couple of days, and on the fifth day a differential blood count revealed 15% monocytes.

When a few drops of broth culture were instilled into the conjunctival sac of a rabbit, conjunctivitis occurred together with a slight swelling of the lids on the fourth and fifth days, and then disappeared. Keratitis did not occur although pus collected at the inner canthus.

An agglutination test employing this organism as antigen (Somatic type), prepared according to Paterson (13) and tested against a known Type I Antiserum, gave agglutination to a titre of 1:60.

Tissues from two additional Arctic foxes, forwarded from Fort Churchill 5 February, 1955, and examined at Kingston, have not yielded *L. monocytogenes* to date.

THE ISOLATION OF L. MONOCYTOGENES FROM LEMMINGS DYING FOLLOWING TRANSFER TO KINGSTON

Following the survey at Fort Churchill, 12 lemmings and 4 voles were brought by air and train in 22 hours. The animals arrived on 1 October in seemingly good condition. On 6 October one was found dead, without any previous signs of illness. Nine lemmings died during the following 10 days, the remainder by 3 January, 1955.

Clinical Manifestations

These signs were sudden in onset. The first sign of trouble was death. Some of the later deaths were preceded by illness of 24 - 48 hour duration. The manifestations of illness were lack of activity in hitherto very active animals, which sat very still in a tucked-up position, hair ruffled, and often with the head in a corner. When they moved, they circled in one direction. The noise of opening the cage was sufficient to

cause convulsions, and in one instance death was observed. The over-all symptomatology was identical with that described by Barrales (3).

Rausch (4) observed a lemming crash in Alaska and noted "that dying lemmings observed on the surface of the snow usually manifested considerable spasmodic or convulsive activity just prior to death".

He also reported "that there were no *grossly* visible lesions such as might be expected from bacterial infections". This is a common observation in Listeriosis.

Bacteriology

L. monocytogenes was isolated from two of the lemmings.

A rabbit injected intravenously with 1.5 ml of whole culture from one lemming became ill and died on the nineteenth day.

When a blood count was made on the eighth day, the monocytes constituted 20% of the total white blood cell count.

Histo-pathology

Eight lemmings that died during the first 10 days and 2 voles showed liver lesions. These consisted of necrotic foci, infiltrated with polymorphonuclear leucocytes, through all the lobules. The livers of one lemming and of two voles which died later showed marked fatty degeneration. No lesions were noted in the heart.

In the brain of two lemmings, extreme purulent leptomeningitis was seen. The lesions were far more marked than in the animals killed at Fort Churchill. In addition, one showed a large intracerebral abscess. The medulla oblongata was not sectioned, having been used for the bacteriological examination.

In one of 3 spleens examined, focal necrosis was observed.

There was marked congestion and slight edema of the lungs in 2 animals.

DISCUSSION

The facts that Listeriosis is recognized as a disease entity practically all over the world and that *L. monocytogenes* has been isolated from many different animal species including man would make one expect it to be of common occurrence. Such is, however, seemingly not the case. Little definite information concerning its transmission and reservoir exists, and the question regarding the mechanism which initiates the infection in certain individuals is still open to speculation. That the conditions which are necessary for its inception are very exacting is a foregone conclusion.

Murray (14) doubts the supposed infrequency of Listeria infection. He points out that its apparently rare occurrence may be due more to its insidious nature and difficulty in its recognition, clinically as well as in the laboratory. It seems highly probable that this is so. Latent infection has been recognized in the captive fox (16) and in the ferret (17). In man and in several animal species, *L. monocytogenes* has been isolated from the aborted fetus and from the newborn (granulomatosis infantiseptica in infants) (18), and from the mother and dam without the latter themselves showing signs of infection (19).

Consequently, the idea that Listeriosis in a latent or subclinical form may be a frequent occurrence in not difficult to accept. The more difficult task is to explain the mechanism of the activation of the latent, insidious infection into an acute, highly fatal one. It would seem, furthermore, that even in initial acute infections this organism demands extremely exacting conditions to be present in the host.

It was already noted that a disease diagnosed as diabetes mellitus occurred in the lemming colony at the Defence Research Northern Laboratory in 1950. The absence of sedges and willow branches from the diet was suspected of being influential to some extent in bringing about this condition (10).

It is interesting to note that the root and bark of the willow tree contain the alcoholic glucoside salicin (20) which

has a pharmacological action similar to phlorizin (21), a glucoside found in the root and bark of apple, cherry, and other trees. These glucosides by their action interfere with the re-absorption of glucose from the glomerular filtrate. At the same time, a disinfectant action is attributed to salicin.

Nearly everyone is acquainted with the belief that the lemming every few years make suicidal migrations, during which these little animals supposedly march into the ocean (7). However, it should be noted that during lemming crashes, many dead and dying lemming are observed on the ground (4). Many may die underground (6).

None of the hypotheses evolved to explain the cyclic crashes of the lemming and of many other wild animal species has withstood a critical evaluation.

A recent discussion of population fluctuation in the vole, *Microtus agrestis*, theorizes "that at the time of approaching peak numbers, strife within the population resulting from competition for some agent such as space results in a physiological disturbance in the majority of adult animals in the population. Offspring produced by these disturbed animals are not viable, that is, these young die during the first year of their lives . . ." (22).

Christian and Davis (23) have shown that "adrenal weight may be decreased and maintained at decreased levels in a population by reducing the population to submaximal levels, presumably because of decreased stress".

If it can be assumed that adrenal activity is proportional to the variation in the size of the gland, it may well be that these observations are extremely important links in the chain of circumstances responsible for the periodic decimation of many wild animal species.

Returning for a moment to our speculation on the pathogenesis of Listeriosis, *L. monocytogenes* is a glucose fermenting organism, and its dependence on glucose for maximal growth is well established.

We have demonstrated that the optimal in-vitro growth of this organism occurs in concentrations which correspond to those found in the blood in the hyperglycemic state, with the optional level at a concentration of 160 to 180 mg per 100 g.

It would seem reasonable to assume that the same level of glucose might also be the most favourable for *L. monocytogenes* in the in-vivo host-parasite relation. It is well known that diabetics are more susceptible to infection with certain bacterial species than are non-diabetics. There seems to be no reason why Listeria organisms should not prefer the borderline glucose-level between the normal and the frankly abnormal.

Another observation lends support to the hypothesis of a predisposing hyperglycemic state in the pathogenesis in this infection. The lesions of Listeriosis observed in the different species are present largely in one or more of the following organs; liver, heart, brain and in the maternal and fetal placentae, and not uncommonly the adrenal gland. Glucose, fructose, and galactose pass from the intestine into the blood of the portal vein and are deposited in the liver as glycogen. The liver is constantly concerned with the processes of carbohydrate metabolism, and might offer the organism its optimal sugar requirements at all times. A large part of the lactic acid produced in the body diffuses into the blood and is converted to glycogen in the liver as well as in the muscles, especially the heart muscles, and galactose is present in the brain as a cerebroside. In the rabbit, gestation produces an impairment of the tolerance to glucose (24). Cortisone administration results in high placental glycogen levels, particularly in the fetal placenta (25). It is permissible to wonder if it might not be more than accidental that these organs with an extremely active sugar metabolism are also those in which the etiological agent of this infection localizes and produces its characteristic lesions.

The level of glucose in the blood may be raised during periods of physical exertion and under conditions of stress, cortisone and other adrenal cortical hormones or adrenalin inducing the mobilization of glycogen. Also, the pituitary gland stimulating the adrenal gland may be instrumental in raising the blood sugar level. These are the conditions one would expect during population peaks in consequence of increased competition. The lemming is an extremely excitable little animal, but highly courageous and ready to fight at any time and against any odds.

In this connection it should not be forgotten "that in the domestic animals listeriosis usually occurs in the winter and early spring, a time when the sheep or cattle are confined in barns for all or part of the time. Losses subside and the disease disappears after the animals are placed on pasture. These facts indicate that crowding has much to do with the transmission of the disease." (10). They may also indicate that crowding has much to do with the occurrence of the disease. Important also is the fact that the birth rate is high during late winter and early spring.

Along with the consequences of crowding which have already been discussed, the consequences of an intense feeding program carried out at the same time might add to the unfavourable conditions as far as the maintenance of health is concerned.

It is of interest that three of four ovine cases reported in the literature (10) in which blood sugar analyses were performed, showed a hyperglycemia, while of three cases in cattle (26) where urinalysis was performed, 2 gave a negative test for acetonemia (hypoglycemia) and the third gave an initial negative test and a later positive test. This latter finding seems significant.

It is, of course, not implied that every case of Listeriosis, whether in man or in any of the domestic or wild animal species, depends upon a hyperglycemic condition for its occurrence. It is theorized, however, that in the activation of a latent infection into an acute fulminating one, a high blood-glucose or tissue-glucose level may be a deciding factor. Once an active infective process has been established in one or more highly susceptible (hyperglycemic) individuals, with an attendant rapid multiplication of the invading organisms and an increase in virulence through a subsequent host passage, the state is set for an extensive epidemic, especially in a population conditioned by some form of stress.

Neither is it implied that *L. monocytogenes* is the only organism which may play a part in the cyclic population declines of many wild animal species, but it seems highly probable that it may be playing a part in the cyclic crashes of the lemming population.

From the evidence presented, it may be speculated that an upset carbohydrate metabolism, whether initiated by stress (over-population, anxiety, fatigue, concurrent disease, gestation, etc.) or by a dietary imbalance, is the trigger mechanism which makes conditions favourable for the proliferation of *L. monocytogenes*.

One aspect of the foregoing is in the use of Arctic animals for emergency rations. The probable existence of *L. monocytogenes* infection in sledge dogs should not be overlooked, partly because of their close co-habitation with man, partly because of the periodic decimation of the dog population by epidemics of uncertain etiology (9). The sledge dog still plays a vital role in life in the north.

SUMMARY

1. The findings of a survey of the lemming colony at Fort Churchill is reported. *L. monocytogenes* was not isolated.
2. The isolation of *L. monocytogenes* from lemmings following their transfer from Churchill is reported.
3. The isolation of *L. monocytogenes* from a seemingly ill Arctic fox

caught at Fort Churchill is reported for the first time.

4. The writer believes the trigger mechanism in Listeriosis may be physiological and that an upset carbohydrate metabolism may be involved.

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