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A CHARACTERIZATION OF LISTERIOSIS IN MAN AND OTHER ANIMALS*

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THE WORLD-WIDE DISTRIBUTION of listeriosis and the features of epizootics in domestic animals, with the almost total lack of knowledge of its transmission and maintenance, give this disease a measure of importance. Human listeriosis has, up to now, been considered sporadic, but recent evidence from Germany gives it a much more momentous incidence and character. The variety of susceptible hosts, with the clinical and pathological variations they exhibit, brings into prominence problems in comparative pathology of striking interest, but, because the processes involved are largely unknown, their true significance has not yet been appreciated. The organism itself, Listeria monocytogenes, presents unusual characters, not alone because it lives up to its name in certain hosts, but because of its tolerances, its liability to be overlooked or confused with other kinds, and, not the least, because of its insidious behaviour. Thus, this disease and its causative organism present an unusual variety of possibilities for research, which might well not only clarify the significance of listeriosis but also develop new approaches to problems in pathology, bacteriology, immunology and biochemistry. These would sequentially enlighten clinical and epidemiological concepts and practices.

Listeria monocytogenes has been isolated and identified in a minimal list of 27 species of animal (rabbit, hare, guinea-pig, gerbille, lemming, mouse, rat, hamster, chinchilla, vole, sheep, goat, cattle, swine, horse, fox, dog, ferret, raccoon, chicken, canary, duck, goose, eagle, capercailzie, unspecified birds and man) and the variety indicates that yet more will be revealed in time. Most instances are in domestic or captive animals, but that certain hosts are abundant wild species, of which some are predatory and others migratory, indicates that the disease should be more widely sought in mortalities of wild fauna. That certain of these may contribute to its introduction to herds is a real possibility, especially since some studies conclude that in domestic animals it is most commonly a disease of winter and spring, when the animals are confined. Evidently the incidence and distribution of Listeria monocytogenes in wild life deserves more attention than it has received.

Migratory and predatory habits, especially the devouring of carcases, seems worth some attention. These, each in their own way, may contribute to the seemingly universal distribution of listeriosis which has been reported from 26 countries in five continents, ranging from the Arctic to the tropics. The list comprises: Argentina, Australia, Austria, Brazil, Canada, Cuba, Denmark, England, Finland, France, Germany, Holland, India, Italy, Japan, New Zealand, Norway, Palestine, Poland, Russia, Scotland, South Africa, Sweden, the United States and Uruguay. In contiguous countries from which it has not yet been reported adequate investigation will no doubt reveal its presence.

A disease with so widespread a distribution, implicating such unrelated and varied hosts, involving widely differing food and living requirements, and, apparently, every kind of climate, must present features and conditions of singular interest.

The clinical, pathological and bacteriological characters of listeriosis vary individually and collectively in different hosts and there is no indication whatever that this is due to differences

in species, variety or type of the infecting bacterium. The species character of the microorganisms isolated from all kinds of host in every country seems clearly to be homogeneous and identical in essential ways, with the exception only that there may be different serotypes involved in different regions and that individual strains may show greater or lesser degrees of virulence to laboratory animals. The serotypes, of which there are four, are not in any way restricted to a particular host and do not show any correlation with clinical, pathological or other features of the disease. These variations are peculiar to the host and are not influences imposed by the strain of *Listeria* involved. This is illustrated not only by independence of the serotype, but also by the finding that strains isolated from cases without monocytosis in the blood, as seems to be the rule in cattle and common in man, nevertheless cause a characteristic monocytosis in rabbits. The same situation is clearly indicated by focal necrosis, which is very marked in rabbits and extensive in the human fetus or infant and is not a feature in the lemming, yet a lemming strain produces the usual necrosis in a rabbit. It is important to recognize this situation since it precludes a too stereotyped clinical description and expectation; it also abolishes any trace of justification for the creation of species of *Listeria* with the epithets "cuniculi," "hominis," "bovina," "ovis" and so forth.

It is not my purpose to give a categorical description of the disease in every species of host, but some examples of contrasts and similarities will emphasize where the human form fits in the general scheme. A meningo-encephalitis dominates the disease seen in cattle, sheep and goats with lesions of the white matter of the brain in addition to varying degrees of meningitis, and the organism is most readily isolated from the brain and especially from the medulla oblongata. Septicæmia is less common. In rabbits and guinea-pigs it is a generalized infection with focal necrosis, especially in the liver and suprarenals, and though isolations can be made from various organs they are very rarely successful from the blood. A feature of natural disease in rabbits is œdema and extensive serous exudates in which triple-phosphate crystals abound. In fowls and at times in guinea-pigs there is massive myocardial necrosis with great numbers of bacteria in the lesions. In some instances it is simply a generalized infection and this in the lemming seems not to be accompanied by any very obvious lesions, even though fatal. In the dog and the fox it is described as a distemper-like disease.

Infection is not of necessity fatal nor may it obviously cause illness, as is instanced by the isolation described in ferrets, in which the condition was almost a carrier state. An instructive report from Finland showed that the toxicity of aureomycin given to guinea-pigs by mouth was due to the consequent development in them of typical fatal listeriosis. *Listeria monocytogenes* seems to have been naturally present in the guinea-pigs and was proved to be very resistant to aureomycin, which seems to have favoured its multiplication in altering the intestinal flora. The situation was controllable with penicillin or chloramphenicol, to which the *Listeria* was sensitive. This insensitiveness to aureomycin is not in agreement with other reports.

Listeria monocytogenes has been isolated from the female genital tract of rabbit, guinea-pig, hare, horse and cattle, and metritis has been described in these animals, as well as mastitis in a cow. Abortion due to it has been described in sheep, goats, swine, rabbits and cattle. The fetus in some of these showed extensive necrosis and cases have also been described of death supervening relatively few hours after birth. Attention was drawn to the similarity of these to human cases in newborn infants and aborted fetuses. Another specialized and peculiar lesion in animals is kerato-conjunctivitis but the susceptibility to it is mostly experimental in rabbits, guinea-pigs and hamsters, while dogs and to a lesser extent sheep and cattle are resistant; natural conjunctivitis has been found in horses and man and it developed in an intracerebrally inoculated cat. This Anton's reaction in rabbits and guinea-pigs is considered to be a singular character of *Listeria monocytogenes*.

In several recent papers it is stated that human listeriosis is a rare disease and some twenty published cases are listed. The true picture is somewhat different. German papers list some 150 described cases and those of us interested in the subject know of a number that have not been published. The German papers go further and review published cases of meningitis, mononucleosis, pseudotuberculosis and granulomatosis, and find 36 cases dating from 1891 providing strong enough evidence to suspect them of

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being listeriosis. As diagnosis depends on identification of the causative organism, whose isolation is none too easy in many instances, it is highly probable that human listeriosis is more common than is realized.

Of the published human cases the category incidence is roughly 33% meningitis or encephalitis, 29% "granulomatosis", 21% septicæmia, 8% mononucleosis and 6% conjunctivitis, leaving out the decimals and making some allowance for overlapping states and imprecision. These are conditions that have been classified, but it is more than probable that other states will be described when the disease is more widely recognized. My reason for this statement is that the condition distinguished by Potel as "granulomatosis infantiseptica" is of recent discrimination, although it is now evidently represented among previously recognized cases of listeriosis and even reasonably suspected among cases described before Listeria monocytogenes was known.

Meningitis, septicæmia and conjunctivitis are straightforward and need no further elaboration, other than to admit that the isolation of the organism is often difficult and to urge that the possibility be borne in mind. The relation of Listeria to mononucleosis is not yet decided and it is probably not the common cause of cases generally so called. Though various authors have isolated Listeria from 12 typical cases of "infectious mononucleosis" it must be remembered that a monocytosis is not a marked feature of authentically identified human listeriosis.

"Granulomatosis infantiseptica" is an intrauterine infection with a high mortality for the fetus or the newborn child. It is a generalized infection of the child with extensive focal necrosis, especially of the liver and sometimes the lungs; there may be meningitis too, but there is commonly little difficulty in isolating Listeria from fatal cases. In less severe cases blood, cerebrospinal fluid, urine or local secretions may not always yield positive cultures, but Potel has made the important observation that the meconium is always positive and often shows abundant characteristic Listeria microscopically. This is thought to be due to swallowing the infected amniotic fluid, and the severe lung lesions are also thought to be due to inhalation of heavily infected liquor. Often the infants die within two or three days after birth and at times with longer delay. Listeria has frequently been isolated post partum and sometimes ante

partum from the mother's vagina and occasionally from her urine or even blood. In some cases the mother shows no sign of illness but in others she has sudden high temperature with rigors, may have pain in the loins or diarrhœa, and bacteriological investigation becomes essential to make an early diagnosis and institute suitable treatment. Though the mortality is high in the infants it is very low in the mothers, which is rather surprising. A rising agglutination titre may be found in the mother and to a lesser degree in the infant.

The pattern of the disease and the lesions seem to be much the same in the fetus or newborn of man, sheep, cattle and rabbit and there is no evident reason to expect it to be otherwise. In so far as bacteria are concerned, man is not a superior being but just another mammal and listeriosis shows no indication of peculiarity to one species with occasional transmission to another. Listeria monocytogenes is a pathogen with a very wide range of hosts and the variation in manifestation is determined by selective peculiarities in the pathological responses of different species of host to the activities, products and antigenic components of the bacterium.

Human listeriosis can no longer be regarded as a sporadic accident in a disease of animals transmissible to man. In many cases there is no indication of an animal source of infection; that Potel has collected some forty cases of "granulomatosis infantiseptica" in the region of Halle (Germany), while several cases in other places conform to his description, suggests that no preconceived restricting concept should be entertained. Although the brunt of the incidence in an outbreak may fall on the young, in terms of mortality or obvious morbidity, nevertheless the infection or carrier rate in the adults at such a time is entirely unknown and, despite inherent difficulties, examination of the gut and urogenital tract might prove informative.

The clinical features of listeriosis are not so well defined that a diagnosis can be made on the case history, signs and symptoms. A shrewd suspicion may be awakened by awareness of prevailing recognized cases but the actual diagnosis depends upon alertness of the bacteriologist. Absolute dependence can only be placed on the isolation and identification of the organism. To this end, cultures should be made from the blood, cerebrospinal fluid, urine, meconium, placenta, lochia, milk, exudates and pus, according

to the indications of the case and means of collecting specimens. At autopsy, cultures should be made from all and sundry organs and sites. Brain tissue, especially the medulla oblongata, must not be neglected and the technique of Gray, Stafseth and Thorp should be used, by which the triturated brain tissue is kept at 4° C. for a week or more and subcultured from time to time. Even in generalized infections blood cultures are often sterile, and this finding should not be accepted as exclusive of listeriosis.

Since the agglutination titre fades rapidly after recovery it cannot serve reliably for late diagnosis, but a high titre (over 1:200) or better a rising titre is significant. This test should be done with "H" and "O" antigens of the four serotypes of Listeria monocytogenes and the epidemic strain, should such prevail. Complement fixation tests have proved helpful only in skilled hands. I am informed that isolations are made in Russia by inoculating animals, but most laboratory animals are susceptible to the natural disease and the experience reported from the Helsinki (Finland) laboratory does not encourage reliance on the method.

Prevailing information gives the impression that listeriosis has a low incidence with a high case mortality, with severe lesions or no obvious lesions according to the species. This statement is open to some doubt because of the difficulty in isolating Listeria, even from the sick. The wide geographical distribution in a great variety of hosts, with a remarkable constancy in the characters of the organism from all these sources, indicates a cryptic persistence and peculiar host-parasite relations, all of which make it difficult to accept the concept of a low transmission rate. It may be merely that individual susceptibility and resistance can account for the situation. The hypothesis of a common source of infection is not prepossessing because of the range of countries, climates and fauna associated with the disease. It seems more likely that a wide host tolerance allows of a catch-as-catch-can spread, using any and every opportunity.

The state of susceptibility can be altered by circumstances, and disease rates may be determined by environmental conditions. The time of weaning of rabbits is a danger and outbreaks in domestic animals seem to have some association with times of confined feeding or radical change in management, but terminate or do not occur when the animals are grazing. Subsidence of the disease has been associated with change of diet, although purposeful experiments have not given convincing results, but epidemic conditions are hard to provide.

As might be expected, outbreaks in herds have been associated with introduction of animals from an infected source, but contamination of food and water is not effective. Whether or not small wild rodents could effect contamination has not been determined; rats have been suspected but the incidence of Listeria in wild animals is too little known to interpret. Transmission by blood-sucking arthropods has been suggested without supporting evidence.

Attempts have frequently been made to relate human cases to contact with domestic animals or use of their unsterilized products and some doubt has been cast on the effectiveness of ordinary pasteurization of milk for Listeria. There are reported instances when the probability is high and it would be strange if it did not occur, but, for the most part, support is lacking for frequent direct infection of man from animals. There are those who emphatically deny the possibility of human-to-human infection, but no reason for this strong opinion is given. At present there are no statistical indications one way or another, and, considering the potentialities the history of listeriosis reveals, mere speculation is of no moment.

Some attention should be given to the unusual resistance Listeria exhibits to ordinarily untoward circumstances. It grows slowly but profusely at temperatures between 2 and 5° C., at which it maintains certain antigenic qualities quickly lost at 37° C. It has been proved to remain viable and fully virulent after eight weeks in 20% NaCl at 4° C. At room temperature and at 4° C. on culture media it survives without loss of virulence for three or four years, so too in brain suspensions, but survival is short in distilled water or normal saline. It has been shown to survive from 6 to 26 weeks in animal feed pellets, hay, straw and wood shavings inoculated with culture. However, it has not been found in litter, feed, straw or drinking water from infected premises, which entails separation from among a large number of other organisms. It has been shown to survive five minutes at 80° C. and one author claims it withstands 15 seconds at 100° C. These observations contribute to the difficulty of envisioning the epidemiology of listeriosis.

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Although there are discrepancies in the estimation of sensitivity of *Listeria* to various antibiotics and sulphonamides, good therapeutic results have been reported with many, except streptomycin. Resistance to streptomycin is easily developed.

The characters of *Listeria monocytogenes* are well described in books of reference, but confusions are not infrequent. Some authors persist in putting this organism in the genus *Erysipelothrix*, and this is even to be found in a book so well accepted as Topley and Wilson. The differentiation of the two genera is clear. The organisms resemble one another morphologically except that *Listeria* is motile, with peritrichous flagella, and *Erysipelothrix* is not. *Listeria monocytogenes* grows well at 4° C., hydrolyses esculin within 24 hours, produces catalase and causes keratoconjunctivitis, but *Erysipelothrix* does none of these things. These differences are confirmed by good authorities and the comparison of 60 strains of *Listeria* with 127 animal strains of *Erysipelothrix rhusiopathiæ* in our laboratory supports them fully. In addition there is no antigenic relationship between them and they differ in pathogenicity. *Corynebacterium* is another line of confusion and some publications, subsequently corrected, placed the organism from the cases described in that genus. Differentiation by motility is not so strictly useful since some motile plant pathogens and soil forms have been called *Corynebacterium;* but growth at 4° C. and tolerance of more than 6% NaCl and antigenic characters are valid.

There are four recognized serotypes established by Paterson based on the "H" antigens of *Listeria monocytogenes,* and in these the "O" antigens are distinctive for Types 3 and 4 but identical in Types 1 and 2. As far as is known at present, Types 1 and 4 are of world-wide distribution but Type 3 seems only to occur in Denmark and Eastern Germany and Type 2 in Great Britain. Although there is no limitation of any serotypes to species of host or form of disease, their recognition is important in establishing the identification of *Listeria monocytogenes* and in epidemiological studies. For this latter purpose further help is available in the recognition of biotypes within the serotypes 1 and 4, by their ability or not to ferment melicitose; as the test is extended by Seeliger, the majority of Type 4 are positive and the majority of Type 1 are negative. These differentiations cannot be

neglected in studies of the clouded field of the source of infection and spread of listeriosis in man and animals.

The monocytosis produced by infection in certain hosts is brought about by a chloroform soluble lipid easily extracted from cultures or from the necrotic livers of animals which die of listeriosis. The lipid is not antigenic and not evidently toxic and occurs in strains from animal species which do not produce a monocytosis as a feature of their illness. It is interesting that rabbits can be maintained in a high state of monocytosis (2,000 or more monocytes per c.mm. of blood) by repeated injection of the extracted lipid, and these animals produce 4 to 16 times more antibody than do normal rabbits to unrelated different kinds of antigen. The antibody can be extracted from the collected monocytes of both actively immunized and passively immunized animals. In view of this it is strangely curious that human cases of listeriosis do not produce higher agglutinating titres in their serum and that their titre does not persist after recovery. But it must be remembered that a monocytosis is perhaps a rare feature of human listeriosis. In any case a close study of this unusual lipid might be of great interest.

It has not yet proved possible to demonstrate a toxin produced by *Listeria monocytogenes,* and the necrosis that is such a feature in many instances of listeriosis is not accounted for. It is possible the conditions required for toxin production have not been realized, and, though its likelihood is indicated by the character of the lesion, it is also possible that an unknown and peculiar process produces the necrosis. In this regard it is worth noting that death from infection with *Listeria* may supervene in animals such as the lemming, without evident lesions such as necrosis.

This general review of the main characters of listeriosis brings out a feature that may be helpful in investigating the unknown problems in its epidemiology: Listeriosis seems less like a disease transmissible from animals to man than like an infection in which man shares with other animals an equal host susceptibility. This view is chiefly supported by the consistent maintenance of the bacteriological and immunological characters of *Listeria monocytogenes* wherever it is found, by the wide range of its world distribution involving every kind of climate and by the astonishing variety of its susceptible hosts.