COMPARATIVE ASPECTS OF LOUPING-ILL IN SHEEP AND POLIOMYELITIS OF MAN*

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By louping-ill in sheep is currently understood a disease characterized by cerebellar ataxia and disorder of brain and spinal cord functions, lasting about a day in acute infections to some weeks in chronic cases. In the latter type paralysis of one or more limbs is generally present. The mortality in definite clinical cases is high, but animals which recover do not usually develop another attack. The association of the disease with particular pastures is well established, and a large proportion of sheep brought from a locality in which louping-ill does not occur into a louping-ill district are liable to develop the disease.

The first conclusive contribution on the causation of this disease was made by Pool, Brownlee, and Wilson¹ in 1930, and the earlier literature on louping-ill has already been reviewed by Pool.^{2,3} These authors recorded the successful transmission of louping-ill in series from sheep to sheep by intracerebral inoculation with material obtained from the central nervous system of affected animals. The pig was also shown to be susceptible.

FEATURES OF LOUPING-ILL

In continuation of the work of Pool, Brownlee, and Wilson, investigations^{4,5,6,7,8,9} at the laboratories of the Animal Diseases Research Association established that:

1. The infective agent is a filterable virus which is communicable to mice as well as to sheep.
2. The pathological changes in the disease are essentially those of an encephalomyelitis.
3. The virus may be present in an infected sheep without producing typical louping-ill, but blood drawn at an early stage of the febrile reaction, which is a prodromal symptom of this infection, contains the virus.
4. Atypical manifestations may comprise merely a febrile reaction, or the sheep may die without lesions in the brain and spinal cord.
5. Under natural conditions louping-ill is tick-borne, the vector being *Ixodes ricinus* L., which is habitually present on louping-ill pastures.
6. Recovery from infection, either naturally or experimentally produced, results in immunity.
7. The investigation of louping-ill has revealed the presence of another tick-borne infection of sheep. This disease, which has been named "tick-borne fever," is clinically, pathologically, immunologically, and aetiological distinguishable from louping-ill, but it probably aggravates the harmful effects of the latter.

That the causal organism of louping-ill is a filterable virus is now generally accepted (Alston and Gibson,¹⁰ and Czarkowska-Gladney and Hurst¹¹). Hurst¹² infected monkeys, and Findlay and Elton¹³ transmitted the disease to field voles. We possess unpublished evidence that cattle are also susceptible, and may develop the disease naturally. Rivers and Schwentker¹⁴ record that human beings who have come into close contact with the virus of louping-ill may develop in their serum neutralizing antibodies against the active agent, and illness suggestive of louping-ill infection is reported in three such individuals.

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Our investigations into the pathogenesis of the disease in sheep and methods of prevention will soon be published in detail, but it may be stated here that there is a prodromal period in the naturally occurring disease, characterized by a diphasic fever. Virus can invariably be detected in the blood during the early part of the first febrile phase. The second febrile phase usually marks the commencement of symptoms indicative of invasion of the central nervous system, and then virus is generally absent from the blood. Many natural cases of louping-ill, however, are abortive. In some of these symptoms of central nervous system infection appear and pass off, whilst in others the only indication is dullness accompanying the characteristic febrile reaction. Abortive attacks can be diagnosed definitely by detection of virus in the blood during the first febrile phase. This diagnosis is made by intracerebral inoculation of mice with citrated blood. There is also evidence that subcutaneous inoculation with formalinized vaccine, prepared from the brain and spinal cord of infected sheep, produces antibodies capable of neutralizing virus introduced into the general circulation, thus preventing the active agent from invading the brain and spinal cord. This vaccine does not immunize the central nervous system, but extensive field trials show that it affords a considerable degree of protection against the natural infection. These observations suggest that the primary multiplication of this so-called neurotropic virus occurs outside the brain and spinal cord.

COMPARISON WITH POLIOMYELITIS

Since this is a discussion in comparative medicine, it seems appropriate to direct attention to the somewhat similar syndrome of naturally occurring louping-ill in sheep and the course of poliomyelitis infection in man. Of course it is not suggested that the two diseases are identical, but, since the pathogenesis and epidemiology of poliomyelitis are subjects of considerable controversy, the study of louping-ill in sheep may present some noteworthy features to those interested in poliomyelitis. At the outset I would mention that, having no personal knowledge of poliomyelitis, I am greatly indebted to the Report by the International Committee on Poliomyelitis,¹⁵ organized by Jeremiah Millbank of New York City.

The medical pathologist, whose material is man, is drastically handicapped by the limited extent to which experiment on the natural material is open to him. He must take his problems as he finds them, and deny himself the help of almost all the preliminary simplifications which are the essence of the veterinary pathologist's advantage. When one's patient can be used as one's experimental animal, circuitous methods of investigation in determining the pathogenesis of a disease do not require to be adopted. Thus the investigation of louping-ill has been greatly facilitated by the fact that sheep, which are naturally susceptible to the disease, are also suitable experimental animals. Such facilities permit a study of the propagation of the infective agent in its natural host, and enable one to place greater reliance on the conclusions drawn regarding the natural route of infection and the epizootiology of the disease than can be placed on similar studies with an infective agent which has been adapted by artificial methods to an experimental animal. In the case of louping-ill the mouse is a susceptible experimental animal, which we have used mainly for detecting the presence or absence of virus in a given tissue. Fortunately, sheep have been available in which to study the pathogenesis of the disease. It has not been necessary to make these studies on the mouse and to argue from the analogous disease in that animal as to the nature of the infection in sheep.

AETIOLOGY AND SYMPTOMATOLOGY

Each disease is caused by a filterable virus which possesses neurotropic characters. Schwentker, Rivers, and Finkelstein¹⁶ showed that immunologically louping-ill and poliomyelitis are not closely related. Incidentally, it might be noted that the poliomyelitis virus used in their experiments was the "mixed strain" virus highly adapted to the monkey. Now it appears from the work of Burnet and Macnamara,¹⁷ Weyer,¹⁸ and others, that repeated passage of poliomyelitis virus through monkeys causes it to change immunologically, and the recent work of Paul and Trask¹⁹ shows that there is a qualitative immunological difference between a freshly isolated strain from a human source and a monkey-adapted strain. Accordingly, the question of immunological relationship between louping-ill virus and a freshly isolated "human" strain of poliomyelitis virus still remains open. The incubation period of naturally occurring louping-ill is rarely less than six, or more than eighteen, days from the time of infection until the appearance of clinical symptoms. This corresponds with the incubation period of poliomyelitis infection. In the prodromal period of louping-ill infection there is usually a diphasic temperature reaction, and the second febrile phase is accompanied by the appearance of the first symptoms of nervous derangement, if these develop. This corresponds with the so-called "dromedary" type of prodromal symptom described by Draper²⁰ for poliomyelitis.

Virus in the Blood

The virus of louping-ill has been detected in the blood of infected sheep concurrently with the initial rise in temperature, and in most instances a fall in temperature is followed by the disappearance of much or all virus from the blood. The virus of poliomyelitis has not been detected in human blood, but I am not aware of systematic daily examination of blood having been made during the prodromal stage. Arguing from the analogy of louping-ill, it is highly improbable that virus would be present in the blood when the symptoms of paralysis had developed. Flexner and Lewis²¹ were able to show the presence of virus in monkey's blood if large quantities were drawn at the height of the disease. If the monkeys showed symptoms of nervous system involvement at the time the blood was taken these workers were probably fortunate in detecting virus. Leiner and von Wiesner²² also reported success in one out of five tests with blood drawn from monkeys at different stages of the disease. Clark, Fraser, and Amoss²³ were successful in finding virus in the blood of monkeys in one out of ten instances; the important point about this positive result, however, is the fact that blood was removed at a fairly early stage of infection—namely, at the beginning of paralysis on the seventh day following intracerebral inoculation.

In poliomyelitis it may yet be found that, as in louping-ill, virus is invariably present in the blood during the early stage of the febrile reaction, and generally absent from the blood when paralysis has developed. In clinically obvious cases of poliomyelitis paralysis of one or more limbs usually occurs. During epidemics the so-called abortive (Wickman type) and non-paralytic types of the disease have been recorded. The most convincing evidence of the occurrence of abortive cases has been furnished by Paul and Trask,²⁴ who isolated poliomyelitis virus from the throats of two patients during a characteristic minor illness which was not followed by paralysis.

In chronic cases of louping-ill, paralysis of one or more limbs usually develops, and on farms where the disease is prevalent sheep with various types of deformity are

usually encountered. As already stated, the existence of abortive cases of louping-ill has been definitely established by the detection of virus in the blood during a febrile reaction, which was the only clinical manifestation of infection other than dullness and loss of condition; such atypical infections are followed by immunity.

RESISTANCE AND IMMUNITY

It is stated that the low case incidence of epidemic poliomyelitis has generally been taken to indicate that many persons are exposed to the virus without developing the typical paralytic disease. This inference, however, is only an indirect one, and not based on any direct experimental evidence. The analogous evidence in the case of louping-ill is direct, since the specific virus has been detected in the blood of sheep during a minor illness accompanied by a febrile reaction which was not followed by paralysis. Further, in one of our experiments, forty-nine susceptible sheep were exposed to the risk of natural infection for two months: 53 per cent. of the animals died from all causes, and 60.9 per cent. of the survivors had acquired immunity to louping-ill.

As in poliomyelitis one attack of louping-ill usually confers immunity, and in both diseases neutralizing substances develop in the blood of infected individuals. The only reliable method of producing a typical case of louping-ill in sheep is by introducing the virus directly into the central nervous system. Subcutaneous or intravenous inoculation with virus is often followed by an abortive attack, or only a febrile reaction. In some instances, however, the typical disease, with involvement of the central nervous system, is produced. Intranasal insufflation with virus is an unreliable method of producing infection; admittedly, infection can be produced by this route, but if it occurs under natural circumstances it must be very rare. We have now had over 2,000 sheep under experiment without observing any precautions to prevent spread by contact, and we have no evidence to suggest that infection may spread by this means. The evidence on this point in regard to poliomyelitis in man is rather conflicting.

Active Immunity by Vaccine

In considering methods of prophylactic vaccination for the control of louping-ill it was found that the central nervous system is a difficult tissue to immunize. Thus formalized vaccine inoculated subcutaneously does not protect sheep from virus injected into the brain, whereas it does protect against virus introduced subcutaneously. Subcutaneous injection of living virus, when followed by a febrile reaction in the inoculated animal, produces immunity of the central nervous system, but this method of immunization is attended with the risk of setting up the disease. Since, under natural circumstances, the disease is transmitted by a blood-sucking arachnid, and the primary multiplication of virus occurs in the blood, it was considered that the immunity afforded by the formalized vaccine might be sufficient to prevent the natural infection. As already stated, although this vaccine does not produce immunity of the central nervous system, it is proving to be efficacious in the control of the natural disease.

Monkeys can be immunized against poliomyelitis by the subcutaneous inoculation of living virus. As in louping-ill the method is attended by the danger of setting up the disease. To quote one example, Leiner and von Wiesner²² inoculated four monkeys; one died of typical poliomyelitis after four injections, one developed the marantic type of the disease, and two died of an intercurrent infection. Success has not attended the efforts to produce immunity by means of dead virus

vaccines. From the analogy of louping-ill this is not surprising, since the immunized animals were tested by intracerebral inoculation.

PATHOLOGY

The essential pathological changes in the two diseases are closely similar, and are mainly confined to the central nervous system, where inflammatory changes occur with destruction of nerve cells, mainly the anterior horn cells of the cord in poliomyelitis, and the Purkinje cells of the cerebellum in louping-ill. From the comparative point of view it is essential to draw attention to the fact that, although the sheep, mouse, and pig are all susceptible to louping-ill, the nature of the reaction in the central nervous system of these animals varies. Thus in the case of the sheep, as the lesions suggest, cerebellar ataxia is the main symptom. In the mouse the nerve cells of the cord are mainly affected, and paralysis of one or more limbs usually occurs; whilst in the pig, which generally does not develop a fatal disease, the interstitial reaction predominates. Even in animals of the same species variation in the extent and location of lesions in the central nervous system may occur, with consequent deviation in the symptoms from those generally accepted as typical.

EPIZOOTIOLOGY

In forming an opinion on the epizootiology of louping-ill it is probably well to bear in mind the insulatory mechanism which guards the central nervous system from injurious substances of haematogenous origin. Wilfred Trotter²⁵ gives a very clear conception of this mechanism. After reviewing evidence, he states:

"We have a group of well-defined facts established by countless observations and capable of confirmation any time. They bring evidence from different parts of the nervous system, disclosing, even to superficial consideration, a clear, common tendency. The conclusion to which all point is that breaches of normal coverings of the nervous tissues, allowing contact between the latter and other tissues of the body, result of necessity in energetic local reactions. The obvious function of such reactive processes is to re-establish the normal discontinuity between neural and somatic tissues, and to break the contact that has caused the disturbance."

McIntosh and Fildes²⁶ showed that after intravenous injection of salvarsan and neosalvarsan in man and animals no arsenic could be found in the brain, although its presence could be detected in tissues outside the central nervous system. This phenomenon is not due to a lack of affinity between the brain and the drugs, but to an inability on the part of the drugs to penetrate into the substance of the brain.

After subcutaneous inoculation of massive doses of potent antitoxin in sheep we have found that although the antitoxin can be detected in the blood its presence cannot be detected in the cerebro-spinal fluid. Whilst it cannot be argued that these substances are comparable to a living virus, the evidence suggests that injurious materials present in the blood have very definite barriers to overcome in gaining access to the central nervous system. It would appear that the epizootiology of louping-ill is closely linked with this fact, and the perpetuation of the disease in enzootic form is dependent on a high case incidence of the so-called abortive form, with a relatively low incidence of clinical cases—that is, cases in which virus has invaded the central nervous system.

This aspect of the problem may also have some bearing on the explanation of the epizootiology and epidemiology of other diseases caused by so-called neurotropic viruses. It is a practical observation that a large proportion of

the old sheep on a "diseased" farm possess immunity to louping-ill, although they have no history of a clinical attack of the disease. Even in a group of yearling sheep received from diseased farms, Pool, Brownlee, and Wilson found that 20 per cent. were immune, and in our own experiments it was found that after exposure of susceptible sheep to the risk of natural infection for two months as many as 60.9 per cent. of the survivors had acquired immunity.

Endemic poliomyelitis occurs in its greatest incidence in children up to 5 years of age. It has an inexplicable seasonal incidence, and is generally regarded as a summer disease. Enzootic louping-ill is generally confined to lambs and yearling sheep; it also has a seasonal incidence, occurring in the spring, and, to a lesser extent, in the autumn. The seasonal incidence of louping-ill corresponds with the season of maximum activity of ticks, which are the vectors of the disease. When an epizootic of louping-ill occurs in a sheep stock in which the disease has not been previously recognized, animals of all ages are liable to become affected. Similarly, epidemics of poliomyelitis in virgin soil have the same tendency. In the International Committee's Report three virgin soil outbreaks are described—one upon the island of Guam in 1899, reported by Grunwell; one upon the island of Nauru in 1910, reported by Müller, and one in New Guinea in 1929, reported by Macnamara. Each outbreak was characterized by a high mortality, mainly confined to young adults. An extraordinary feature common to each outbreak was the apparent immunity enjoyed by the children. This is a peculiar phenomenon, and might suggest that the incidence of subclinical cases in children is greatly in excess of frank cases, with definite involvement of the central nervous system, whereas the same may not apply to non-immune young adults.

Among the many opinions on the epidemiology of poliomyelitis the one expressed by Lavinder, Freeman, and Frost²⁷ is of particular interest. They state that

"while the incidence among a population affected by poliomyelitis even in its severest epidemic form is usually not high, yet this disease possesses not infrequently the power to spread widely—in fact, to become pandemic in a country. It has been suggested that an epidemic of infectious disease may be viewed as the resultant of two excursions, a vertical one representing the heaping-up of cases in any locality, as usually shown plotted on a chart, and a lateral one, representing the extent of territory covered—the geographic distribution. Epidemic diseases not infrequently show decided variation in these two movements. Certain of them, like dengue, for example, show a most striking vertical movement along with a very limited lateral movement. Poliomyelitis has exhibited a significant power of lateral movement, and, up to the present time, in much greater degree than its power of vertical excursion."

This "creeping tendency," as Dale²⁸ calls it, is suggestive of a limiting factor of host resistance, a sparse but widely spread population at risk, and a widespread virus. This opinion on the epidemiology is compatible with the view that poliomyelitis may be a systemic infection with the specific virus which, under favourable conditions, successfully invades the central nervous system, and is responsible for the symptom-complex of poliomyelitis. Such a definite view would materially assist in solving many of the baffling problems in the epidemiology of poliomyelitis.

In view of the modern trend of opinion (Walshe, Annual Meeting of the British Medical Association, 1933) that poliomyelitis is probably from the outset and exclusively an infection of the nervous system, it may be of comparative interest to draw attention to the fact that in an analogous disease of sheep—namely, louping-

ill—there is a high infection incidence of the young population, a large proportion of the cases being sub-clinical infections which can only be diagnosed by detection of virus in the blood, and a small proportion of frank cases with symptoms indicative of central nervous system infection. Thus, in the perpetuation and spread of this disease systemic infections probably have a more important role than actual clinical cases.

SUMMARY

Louping-ill, an encephalomyelitis of sheep, is caused by a filterable virus possessing neurotropic characters. Under natural conditions of infection the disease is tick-borne, the vector being *Ixodes ricinus* L. At first it would seem a curious anomaly that a blood-sucking arachnid has the role of transmitting a neurotropic virus, but the apparent anomaly is explained by the fact that multiplication of this so-called neurotropic virus occurs primarily in the blood. Invasion of the central nervous system by the virus occurs at a late stage in the infection, and is responsible for the characteristic symptoms of the disease. In the naturally occurring disease, invasion of the central nervous system is not a constant feature. Cases occur in which virus does not gain access to the central nervous system. Such abortive types of the disease can be diagnosed by the detection of virus in blood drawn at an early stage of the febrile reaction which accompanies the infection. It seems highly probable that the incidence of abortive types of the disease is greatly in excess of the number of frank cases with central nervous system involvement.

From a comparative point of view the study of louping-ill in sheep would suggest the possibility that in the early stage of poliomyelitis in man, before obvious clinical symptoms have developed, the specific virus may be present in the blood. If such early blood infection were demonstrated present views regarding the nature of poliomyelitis infection, its prevention, and treatment would require revision.

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URINARY EXCRETION OF OESTRIN ADMINISTERED UNDER EXPERIMENTAL CONDITIONS AND AFTER THE MENOPAUSE

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That oestrin is excreted by the human female in the urine and faeces at various stages during active sexual life is now definitely established. The quantities excreted show regular alterations during the menstrual cycle, these variations applying to the amounts found in both the urine and the faeces, and being greatly increased during pregnancy. A less marked increase in the oestrin excretion is also noted during the early stages of the menopause. The fluctuations in the elimination of oestrin correspond in general to changes in the hormone content of the blood. When, however, we come to consider the question as to how much oestrin is actually produced in the body at these different stages, and whether the hormone produced is quantitatively excreted without undergoing any changes, there are few experimental data on which any conclusions can be based. A similar paucity of information exists with regard to the function of the renal and intestinal barriers, and the problem of whether these play any part in determining the excretion of the sex hormone.

Zondek (1931) has, indeed, reported one case in which the elimination of oestrin was studied. The patient was oophorectomized for ovarian tumour, and subsequently the parenteral administration of 10,000 mouse units of oestrin was followed by excretion of 60 per cent. of the hormone in the urine within thirty-six hours. As some of the hormone was presumably also excreted in the faeces, the figures suggest that only a small percentage of the oestrin introduced in the body was retained and not excreted. On the other hand, both Siebke (1930) and Siebke and Schuschania (1930) also investigated the hormone excretion in the urine and faeces following the repeated administration of known doses both parenterally and hypodermically, and concluded that a large proportion of the hormone is *not* excreted, and must therefore, in some way, be used up or metabolized in the body.

OBJECT OF INVESTIGATION

Now knowledge as to the fate of the oestrous hormone is important for a number of reasons—namely:

1. It may help us to understand more perfectly the nature of the sex cycle and its relation to hormonal activity.
2. It will allow of a more exact interpretation of the diagnostic significance of data on the presence of the hormone in the excretions—in relation to the hormone production and activity.
3. It may help in the evaluation of the doses necessary to bring about certain desired therapeutic effects.
4. In view of the great variety of active oestrus-producing compounds now isolated, it may afford information as to which of these may be the most suitable in relation to the normal metabolic processes.