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STUDIES ON A NEW ENCEPHALOMYELITIS
OF SWINE IN ONTARIO

A Thesis
Presented to the School of Graduate Studies
of
The University of Toronto
by
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In partial fulfilment of the requirements
for the degree of
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BIOGRAPHICAL SKETCH

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INTRODUCTION

A disease of suckling piglets, which was characterised by encephalomyelitis, was discovered in Ontario in 1957. In 1958 it reached serious proportions thus predicating the need to determine its nature and cause. With these aims in view the work presented in this thesis was undertaken.

The disease is here called "Ontario Encephalomyelitis of Swine" or "Ontario Encephalomyelitis" to avoid confusion with other porcine encephalomyelitides in later discussion but it is not meant to imply that this is a new disease of pigs.

This thesis contains a description of "Ontario Encephalomyelitis of Swine", a comparison of this with other known procine encephalomyelitides and the details of the investigations of its cause.
"Ontario Encephalomyelitis" was first seen in November, 1957 and it has since then assumed the proportions of a minor epidemic. Suckling piglets under two weeks of age were the most severely affected group. In these, whole litters were frequently affected with the disease usually appearing explosively and without premonitory signs. If other litters of a similar age were present on the farm they were usually involved also. Pigs up to four months of age have been found affected but amongst these older pigs the morbidity was very low. The mortality in young pigs was high and whole litters were often lost. The disease was less severe in older pigs and recovery was more common. In most outbreaks, litters farrowed two to four weeks after the first animals became sick did not contract the disease.

The clinical signs were those of an encephalomyelitis which followed a rapid course. In acute cases the piglets rapidly became unconscious with convulsions and hyperesthesia as the most prominent signs, often accompanied by a rather profuse salivation; in less acute cases depression, ataxia, and posterior paresis were often seen preceding loss of consciousness. Details of the clinical syndrome and hematology have been presented by Alexander et al (1).
Pathology

Material and Methods.

(i) Histology. One hundred and two pigs were autopsied from 61 outbreaks of the disease. All material for histologic examination was fixed in 10 percent formalin, except eyes which were fixed in Zenker's fixative immediately after the piglets were killed. When material was taken for transmission experiments only one-half of the brain was available for histologic examination. If the piglets were dead when presented for examination only sufficient sections were cut to confirm the diagnosis. A more extensive examination was carried out on the central nervous system of 55 pigs killed immediately prior to autopsy. Blocks were prepared from many parts of the brain and at least three levels of the spinal cord in such a manner as to include adjacent spinal ganglia. Sections were stained with hematoxylin and eosin; selected sections were stained with luxol fast-blue, periodic acid-Schiff, phosphotungstic acid-hematoxylin, Cajal's gold chloride method for astrocytes, and toluidine blue.

Sections were prepared from various non-neural tissues in some cases and a particularly extensive examination was carried out on five, acutely-diseased piglets from which the following tissues were examined: esophagus, stomach, jejunum, colon, liver, kidney, bladder, spleen, lymph node, pancreas, parotid salivary gland, turbinate bones, lung, myocardium, aorta, thyroid gland, adrenal gland, skeletal muscle from the lumbar part of the longissimus dorsi, diaphragm, and skin.
(ii) Examination of Cerebrospinal Fluid. Cerebrospinal fluid was collected from the cisterna magna of normal pigs and pigs infected naturally with "Ontario Encephalomyelitis". Immediately after the piglet was killed the dorsal atlanto-occipital membrane was exposed and the cerebrospinal fluid was collected by piercing the membrane with a syringe and needle. A Pandy test and a total white cell count were done on suitable samples. The methods used were those described by Gradwohl (24). If the sample contained blood macroscopically or if erythrocytes were seen in the counting chamber, the sample was discarded. Sometimes it was necessary to confirm the presence or absence of erythrocytes by studying a stained smear.

A Pandy test was done on the cerebrospinal fluid from 27 pigs and a total white cell count was done on 11 of them. These pigs were between four and 14 days of age when killed and each was shown subsequently to have lesions of "Ontario Encephalomyelitis". The cerebrospinal fluid of five normal pigs, ranging in age from 12 hours to eight days, was also examined.

(iii) Bacteriology. Tissues from naturally infected pigs were examined for bacteria by plating them onto tryptose agar* with five percent citrated sheep blood added and onto MacConkey agar*. Cultures were taken from the spleen of 44 pigs, from the small intestine of 38, and from the brain of 25 pigs, all of which were killed immediately before autopsy. Six brains which had been held at -15°C for periods varying from nine to 140 days were cultured anaerobically and were also

* Difco (Difco Laboratories Inc., Detroit 1, Michigan, U.S.A.)
examined for *Listeria monocytogenes* by grinding approximately 0.5 gms of material in 3.0 ml of tryptose-phosphate broth in a TenBroeck grinder and streaking directly on blood agar, MacConkey, and sodium azide blood agar* plates. In addition, the brain suspension was incubated overnight in tryptose-phosphate broth and subsequently subcultured on the above solid media.

Observations

(i) Histology. Characteristic gross lesions were not observed at autopsy. There was a mild catarrhal rhinitis in some pigs, and in others the lymph nodes were congested. Usually the stomach was empty except for a small amount of mucus but in some cadavers there was a large milk-curd frequently mixed with greenish, bile-stained mucus; this was present in naturally-fatal cases and is consistent with the oftentimes rapid course of the disease.

Histological changes were confined to the nervous system and were of the nature of a non-suppurative, non-demyelinating encephalomyelitis of a type which is usual for the viral encephalitides. It was characterised by perivascular cuffing, gliosis, and neuronal changes in the brain, spinal cord and paravertebral ganglia, and by a mild meningitis. Inclusion bodies were not found.

The perivascular cuffs consisted almost entirely of a single cell type which had a rather vesicular nucleus and a moderate volume of

* Difco (Difco Laboratories Inc., Detroit 1, Michigan, U.S.A.)
weakly-acidophilic cytoplasm. These cells resembled primitive reticulum cells and they were interpreted as having developed in situ by proliferation of adventitial cells, rather than as being of hematogenous or infiltrative origin; in the smaller cuffs they were present in the wall of the vessel rather than about it and occasional mitoses were found in the cells (figure 1). The width of the cuffs varied from a single layer of cells to six or more layers (figure 2) and in the broader cuffs there were a few lymphocytes and eosinophils but rarely neutrophils. Other vascular changes were slight. The vascular endothelium was either normal or the nuclei were slightly swollen, but the integrity of the vessels was maintained.

A glial reaction was consistently observed, in which microglia appeared to be the cell type chiefly involved. Both diffuse and focal gliosis were present but were of variable severity (figure 3). The focal gliosis took the form of loose collections of cells, or of dense, tight cell-nodules which frequently showed some central lysis of tissue (figure 4). Necrosis of some glial elements in the centers of such nodules was observed and fragmentation of neuroglia was sometimes found in areas of diffuse gliosis. The glial nodules were one of the most characteristic features of the disease.

Neuronal changes were present but were scattered. Degenerating neurons were shrunken and hyperchromatic, particularly with eosin, and had pyknotic nuclei. Satellitosis was present about some of the neurons and neuronophagia was noted but was not common. In some cases cell nodules appeared to have developed from neuronophagic nodules and in these microglia were the main cells present (figure 5). The perivascular
cuffing and the cell nodules were more conspicuous and characteristic of the disease than the neuronal changes.

The pia-arachnoid usually showed changes of a non-suppurative leptomenigitis which varied considerably in severity. The main cell type was similar to that found in the perivascular cuffs but lesser numbers of lymphocytes and plasma cells were also present. The choroid plexus and ependyma were always normal.

Lesions similar to those in the central nervous system were found in the spinal ganglia and Gasserian ganglia but were seen best in the latter (figure 6). Scattered neurons showed degenerative changes of a type similar to those seen in the brain and spinal cord. Accumulations of mononuclear cells were present, particularly about neurons, and these cells had the appearance of having developed in situ but did not seem to be the ordinary capsule cells (figure 7). Where neurons were degenerating these cells were present inside the capsule and filled the space which had been occupied by the neuron so that dense cellular nodules were formed. In many of these nodules fragments of the degenerating neurons were seen. Small groups of cells were present in the interstitium of the ganglia but these were overshadowed in number by the cellular accumulations about the neurons.

The distribution of the lesions varied somewhat but in general followed a pattern of involvement of the cerebrospinal axis from the olfactory bulbs to the caudal spinal cord with a relative sparing of the cerebrum and cerebellum. At all levels the grey matter was more severely involved than the white matter. The olfactory bulbs from 13 piglets were examined, and changes were found in eight of them. The
lesions had no characteristic distribution and any layer of the olfactory bulb could be affected (figure 8). The frequency and intensity of the lesions increased caudally through the rhinencephalic cortex, the corpus striatum, the hypothalamus, and the thalamus, being always more severe in the midbrain, pons and medulla oblongata and decreasing in frequency and intensity down the spinal cord.

The brain stem was affected to a greater extent than any other part of the central nervous system. The medulla oblongata (figure 3) and mesencephalon usually showed the most severe inflammatory reaction, particularly in the areas surrounding the fourth ventricle and the aqueduct of Sylvius. In the thalamus (figure 9) the changes were quite variable in extent but were frequently as severe as those of the medulla oblongata and mesencephalon. A few cell nodules were present in the optic tracts in a small number of cases. The meninges covering the brain stem were either normal or showed slight cellularity.

The spinal cord was less severely affected than the brain stem. The grey matter surrounding the central canal (figure 10) and of the dorsal horns (figure 11) was usually involved to a greater extent than that of the ventral horns and in some cases no lesions were present in the ventral horns. The distribution was reversed in pigs older than about six weeks of age; in these the inflammation of the spinal grey matter was usually more intense with severe changes occurring in the ventral horns (figure 12). Lesions in the spinal white matter were much less severe than in the grey matter, and much less common; perivascular cuffs were present, especially adjacent to lesions of the grey matter, and occasional loose glial nodules were found. All
levels of the cord could be inflamed but there was a tendency for the cervical cord to be the most severely affected part. In about 10 percent of cases, there was a very mild spinal leptomenigitis. Demyelination was not found.

The cerebellar and cerebral cortices were always less severely affected than the brain stem. The cerebellar peduncles, the dorsal roof-nuclei, and the surrounding regions showed the greatest changes, and lesions were present in these areas in all cases. The molecular, Purkinje-cell, and granular-cell layers were often normal but in some cases mild perivascular cuffs of the characteristic type and scattered, loose cell-nodules were found in the molecular layer and rarely in the other two layers. The white matter extending into the gyri was only slightly affected. The cerebellar meninges showed lesions almost constantly, especially about the vessels in the sulci (figure 13). Usually the meningitis was rather mild, and whereas in other parts of the brain the meningeal changes were usually associated with underlying parenchymal lesions, this was not necessarily the case with the cerebellar meninges where, often, associated lesions in the cortex were not found. In a minority of cases, especially in pigs older than about six weeks of age (figure 14), a more severe meningitis was present but in these it was usually accompanied by lesions in the molecular layer.

The lesions in the cerebral cortex were of the characteristic type (figure 15) but were generally mild; they were absent in approximately 50 percent of the cases examined. When present the lesions tended to be more common and severe in the piriform lobes and adjacent
temporal cortex (figure 16). In a few pigs a meningitis of moderate severity was present over the cortex, particularly about the vessels in the sulci (figure 15), but generally the meningeal inflammation was mild and corresponded with the severity of the parenchymal lesions.

Neuritis of the dorsal-root ganglia was constant. The Gasserian ganglia (figure 6) usually revealed the most striking reaction but the spinal ganglia at all levels were almost invariably involved even though lesions were not observed in the adjacent part of the spinal cord. Other ganglia were collected and lesions were found in the cranial cervical sympathetic ganglia in nine of 10 pigs examined, in the stellate ganglia in one of two, in the thoracic sympathetic ganglia in two of four, in the celiac ganglia in four of seven, in the nodose ganglia (figure 7) in 13 of 13, and in thejugular ganglia in two of two pigs. The lesions in these ganglia were of the same type as those in the Gasserian and dorsal-root ganglia and, while the sympathetic ganglia were affected only mildly, the inflammation of the ganglia of the vagus nerve was generally severe.

Sagittal sections of eyes were examined from 21 affected piglets and lesions consisting solely of isolated glial nodules in the inner plexiform layer were found in two of these.

(ii) Cerebrospinal Fluid. The Pandy test was positive on the cerebrospinal fluid of all naturally-infected piglets which were examined. In the majority of cases the tests gave a strong reaction for protein. The test on the cerebrospinal fluid of normal pigs was negative.
The results of the total white cell counts are given in Table I. An average of 174.7 cells/cm³ with a maximum of 583 cells/cm³ and a minimum of 33 cells/cm³ was found in cerebrospinal fluid of natural cases of "Ontario Encephalomyelitis". A comparison of these figures with those found in normal piglets, in which an average of 48.2 cells/cm³ with a maximum of 74 cells/cm³ and a minimum of 29 cells/cm³ was found, reveals that a mild pleocytosis exists in some cases of "Ontario Encephalomyelitis", while in other cases the number of white cells is within normal limits. At the time of counting the cell type was noted but a differential count was not done. The cells were mononuclear cells; cells of the granulocytic series were not seen.

(iii) Bacteriology. Specific pathogenic organisms were not isolated consistently. A small number of colonies of Escherichia coli were grown from five of the spleens; the other 39 were negative. The 38 small intestines yielded a mucoid E. coli in 11 cases, a hemolytic E. coli in three, and a non-hemolytic E. coli in 13. A few colonies of staphylococci were isolated from two of the brains and a small number of colonies of mucoid E. coli from one; the remaining 22 were bacteriologically sterile. Listeria monocytogenes was not isolated and the anaerobic cultures were negative.
### TABLE I

Total White Cell Counts on Cerebrospinal Fluid.

<table>
<thead>
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<th>&quot;Ontario Encephalomyelitis&quot; (cells/cmm.)</th>
<th>Normal pigs (cells/cmm.)</th>
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<tr>
<td>190</td>
<td>60</td>
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<td>42</td>
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<tr>
<td>33</td>
<td></td>
</tr>
<tr>
<td>187</td>
<td></td>
</tr>
<tr>
<td>583</td>
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| Mean                                    | 174.7                    | 48.2 |
| Maximum count                           | 583                       | 74.0 |
| Minimum count                           | 33                        | 29.0 |
Summary

The disease described here can be characterised as a non-suppurative, non-demyelinating encephalomyelitis and ganglionneuritis. The lesions are typical of those caused by neurotropic viruses and emphasis is given to those in the following discussion of comparative aspects. The nature of the nervous lesions, the absence of associated lesions, and the negative results of cultural examinations eliminates bacterial infections from further consideration.
COMPARATIVE ASPECTS OF "ONTARIO ENCEPHALOMYELITIS" AND VIRAL NON-SUPPURATIVE ENCEPHALOMYELITIDES OF SWINE WITH REVIEW OF LITERATURE

The list of diseases affecting the porcine central nervous system is of considerable length. In general, the bacterial diseases cause a diffuse or focal suppurative lesion which is chiefly a meningeal reaction with less severe parenchymal inflammation. The viral diseases produce non-suppurative changes of a more diffuse nature in the parenchyma and frequently the lesions are characteristic enough in their type or distribution to aid the differential diagnosis. Further identification is made on the basis of host range and serologic properties of the viruses. A review of the known viral encephalomyelitides of swine is pertinent to the problem of establishing the relationships of "Ontario Encephalomyelitis".

Teschen Disease

Teschen Disease was first recognized as an entity in Czechoslovakia by Trefny in 1929 (77) and since then it has been recognized in Germany, Austria, Hungary, Yugoslavia, France, Switzerland (81), Italy (75), and Madagascar (65).

Good clinical descriptions of the disease have been published by Kaplan and Meranze (40), and Jones (39). The former authors reviewed much of the earlier German and Czechoslovakian literature and they recognized three stages in the clinical disease. The prodromal stage usually lasted from one to three days and during this period the
animals might show infrequent vomition, depression, anorexia, sometimes a slight ataxia of the rear limbs, and a slightly-raised temperature of 104° to 105° F. The prodromal stage was followed by the stage of nervous excitement which lasted for two to four days and during this period generalized muscle tremors and convulsions were common. During the convulsions, gritting of the teeth with the appearance of frothy salivation at the corners of the mouth, nystagmus and tonic-clonic spasms of the limbs might be seen, with the animal appearing relatively normal between convulsions. Hyperesthesia was often present during this stage.

The stage of nervous excitement gradually passed into the paralytic stage; however the latter could follow the prodromal stage directly without the appearance of the second stage. The paralytic stage was characterized by a progressive ascending paralysis in which the hind limbs were first and most severely affected but in some cases all four limbs and the muscles of the head and neck became involved. Convulsions could occur during this period but consciousness was usually maintained and in many cases animals attempted to drag themselves about by their forelimbs. The body temperature during this period was usually normal or subnormal and the appetite was good.

Most animals died during the first two weeks of illness without necessarily showing paralysis. Complete recovery occurred in about one-half of the survivors within three to six weeks but in the remainder paralysis persisted for many months and atrophy of the leg muscles occurred.
The disease affected very young or fully grown swine and has been observed in pigs from five days of age to those weighing 200 Kg (43). The age group affected apparently depended on whether the disease was epizootic or enzootic in the district. Where the disease occurred for the first time all age groups were affected but where the disease was enzootic, young pigs and newly-introduced animals suffered most (40). Klobouk (43) believed that the morbidity was about 50 percent and that the mortality was approximately 70 percent. Kaplan and Meranze (40) stated that the mortality was usually 50 to 70 percent but might sometimes be as high as 90 to 100 percent; however they pointed out that these figures varied in different outbreaks, apparently depending on previous experience with the disease in a locality; the overall mortality was highest in piglets under four weeks of age.

A number of authors suggested that Teschen Disease was not very communicable by direct contact but Kaplan and Meranze (40) believed that this was probably due to frequent inapparent infections which left the animals immune to further attacks by the virus. Klobouk (43) felt that the disease in Czechoslovakia was less acute clinically than that observed before World War II, and Jones (39) pointed out that in enzootic areas Teschen Disease assumed a sporadic character with only individual animals on a farm affected.

Many workers have reported on the pathology of Teschen Disease and basically they agreed on the nature of the changes observed histologically but differed on minor points. Perhaps two of the best reports are the recent publications by Manuelidis, Sprinz, and
Dorothy Horstmann (55), and by Karin Fischer (17). The former workers used experimentally-infected animals exclusively, while Karin Fischer used both natural and experimental cases.

Specific gross lesions have not been observed (32) (39) (40). Kaplan and Meranze (40) pointed out that secondary non-specific lesions such as terminal bronchopneumonia and decubital lesions could occur; they drew attention to various authors who believed that lesions such as cerebral edema and congestion, petechial hemorrhages in the brain, mild serous meningitis, conjunctivitis, congestion with small hemorrhages in the nasal mucosa, hyperemia and edema of the lungs, or disseminated pneumonic foci throughout the lungs, gave a guide to the diagnosis of Teschen Disease. These observations were not supported by the bulk of the reports and could not be considered reliable.

There was general agreement amongst authors with respect to the type of histologic lesions but differences were observed in their distribution in the nervous system. Essentially the lesions are those of a poliomyelitis characterized by neuronal degeneration, perivascular cuffing, and glial proliferation.

The changes in neurons were usually seen best in the motor neurons of the ventral horns of the spinal cord. Neurons showed various stages of degeneration and they could be found undergoing chromatolysis, vacuole formation, karyorrhexis, and complete dissolution; frequently the cell body finally shrank to a dark, basophilic, sharply-angular mass (40). Many degenerated neurons were removed by phagocytosis but others, especially those which stained acidophilic with hematoxylin and eosin, might undergo dissolution and be converted into a
fluid-filled cavity (55). Focal and diffuse proliferations of glia were a feature of the disease. Glial nodules and neuronophagic nodules were formed and the main cells forming them were microglia with a few lymphocytes intermingled. The perivascular cuffs were described by Manuelidis et al (55) as consisting mainly of a lymphocytic infiltration with a small number of plasma cells and histiocytes present.

Lesions were most severe in the brain stem and spinal cord. Kaplan and Meranze (40) pointed out that the lesions occurred in all levels of the cord but particularly in the cervical and lumbar parts; however, Manuelidis et al (55) considered that there was no distinct difference in the intensity of changes in the various levels of the cord. There was general agreement that the ventral horns were the most severely affected part of the spinal cord but the posterior horns did not escape. The lesions in the brain stem were essentially similar to those of the spinal cord. The cerebellum showed marked inflammation, especially in the cortex where degenerative changes were observed in the Purkinje-cell layer and many neuronophagic nodules were often present (17) (55). The lesions in the cerebrum were less severe than those of the cerebellum and were of variable intensity (40) (45) (55).

Manuelidis et al (55) found lesions in the ganglia which consisted of neuronal degeneration, proliferation of capsule cells, and infiltration of the interstitium with lymphocytes and a lesser number of plasma cells. Changes were present in the following ganglia: spinal, Gasserian, cervical sympathetic, celiac, stellate, and thoracic sympathetic but the inflammation was mild in the last two. These
ganglionic changes were a feature of Teschen Disease but some authors 
(3) (40) believed that similar changes occur in Hog Cholera. The 
observations of Done and Harding (11) did not support these opinions;
they found in a comparison of the two diseases that the ganglioneuritis
of Teschen Disease was of great value in differentiating Teschen Disease
from Hog Cholera.

Manuelidis et al (55) described a lymphocytic meningitis which
was constantly present. It was most severe over the cerebellum, less
severe over the cerebrum and slight over the diseased spinal cord where
it tended to be focal rather than diffuse. These workers pointed out
that the meningitis was not always related to the degree of cortical
damage. Other authors (40) (44) found that the cerebellar meningitis
was not always as severe as that described by Manuelidis et al and was
of variable intensity.

Although Scheuer (70) reported many inclusion bodies in the
nerve cells, other workers agree that inclusion bodies do not occur
(44) (55).

When studying the development of changes in the central
nervous system Manuelidis et al (55) observed that the brain was
involved earlier in the course of the disease than was the spinal cord
and, whereas lesions could be found in the brain early in the disease
they might be present in the cervical part of the cord only at this
stage or be absent from the cord. They also found that a lymphocytic
meningitis over the cerebellum was one of the earliest manifestations
of Teschen Disease. They were unable to find differences in the
distribution or severity of the lesions in pigs infected by intranasal,
intracerebral, or oral routes. The more recent findings of Karin Fischer (17) do not agree with these results; she found that the lesions in animals infected intranasally and intracerebrally were similar, and in them the earliest lesions were present in the olfactory bulbs, with the disease then spreading over the telencephalon, diencephalon, and brain stem to the lumbar cord; the cerebellum did not become affected until a week or so later. The changes in animals infected intracerebrally and intranasally differed in their distribution from those produced by intramuscular, intravenous, and, in most cases, oral routes. The latter group had a distribution similar to that of naturally-infected animals with lesions absent from the olfactory bulbs and telencephalon and of diminished intensity in the cerebellum.

It is generally agreed that experimental animals are relatively easy to infect by intracerebral, intranasal, subdural, or oral routes. Success has also been obtained with intravenous (17) (60), subcutaneous (43) (60), intramuscular (17) (19), and intraperitoneal (46) routes, but these methods were much less reliable.

The virus is readily found in brain and spinal cord collected during the early days of illness but Fortner (20) could not find it in material taken later than the fifth day. The virus has been isolated from gastric mucosa (40), blood (32), mesenteric and hepatic lymph nodes, tonsil, liver, spleen, kidney, and diaphragm (29) but it appears to occur only transiently in these sites. Feces have been shown to contain Teschen virus (19) (21) (29) but this is not a constant finding. Hecke (29) found it from the first to the eighth days after oral infection but Fortner (20) noted that the feces of acute cases were only
sometimes infective and Horstmann (32) could not demonstrate it in feaces even though large amounts of inocula were given orally and consecutive samples collected during the course of the disease were examined.

The virus has a strict host range, domestic and wild pigs only being susceptible (47) (60). Numerous attempts have been made to infect mice, rats, guinea pigs, and rabbits by various routes but these were unsuccessful (40). Sheep were resistant and subcutaneous inoculation of cattle and calves failed to produce disease (8). Numerous routes were tested in monkeys but without success (32) (50) although Dobberstein (9) reported success with apes. In addition Dorothy Horstmann (32) also tried newborn mice, hamsters, cotton rats, and meadow voles, but she was unable to establish infection. The virus could not be grown in fertile hens' eggs (19) (32) (39). The virus of Teschen Disease did not cause hemagglutination (32) (61).

Multiplication of the virus in tissue cultures of swine kidney with the production of cytopathogenic effects occurred (48) (58) and Mayr and Schwöbel (59) described the changes that took place.

Talfan Disease

A viral poliomyelitis of pigs, named Talfan Disease, was described in England by Harding, Done, and Kershaw (28) in 1957. The disease usually occurred in pigs of suckling and immediately post weaning age, but pigs as old as eight months have been found affected. The clinical signs were those of an afebrile ataxia or flaccid paralysis and, although convulsions were seen, cerebral signs were not a feature
of the disease. It was characterized by a low morbidity and mortality. The incubation period in experimental infections was found to be from 12 to about 19 or 20 days.

The histologic lesions in the brain and cord were essentially similar in character to those of Teschen Disease. Lesions were present in the white and grey matter but were more severe in the latter. The most severe damage occurred in the ventral horns of the spinal cord, especially of the lumbar part, but a severe inflammation was also found in the brain stem. The cerebellum showed cortical damage with a variable leptomenigitis which was sometimes considerable but damage to the Purkinje cells was not striking. There were changes of variable intensity in the cerebrum and meningitis was slight or absent over it.

A ganglion neuritis was noted in the spinal ganglia. Lesions were not found in the celiac ganglia of experimental cases and the sympathetic ganglia of naturally infected animals were apparently not examined. Eosinophils, usually in small numbers, were found in the perivascular cuffs but neutrophils were very rare. Demyelination in the dorsal funiculi was found but it was usually mild.

Talfan Disease appears to differ from Teschen Disease in having less severe histologic lesions and in having a less dramatic clinical syndrome. Although isolation from field cases was not easy (64), a strain of the virus of Talfan Disease was established in swine kidney tissue cultures and Chaproniere et al. (6) were able to demonstrate that the viruses of Talfan and Teschen Disease were immunologically indistinguishable. Done and Harding (11) compared the neuropathology of the two diseases in experimental pigs and they found that the
lesions were indistinguishable. On the basis of the serology and pathology it was concluded that Talfan Disease was a mild form of Teschen Disease which had been in Great Britain for some years. It is worth noting that the disease did not appear suddenly and explosively as would have been expected from the previous reports of Teschen Disease, and it seems that the disease had become widely spread before it was recognized.

Poliomyelitis suum (Benign Enzootic Paresis) of Denmark.

This disease was described in 1955 by Bendixen and Sjolte (4) and subsequently an excellent monograph, describing details of the histopathology and transmissibility, has been published (76). The disease has been found in pigs from three to four weeks of age to those weighing 80 kg but the greatest incidence occurred in pigs between 12 and 16 weeks of age. The disease was chiefly an ataxia, paresis, and paralysis, and cerebral symptoms were observed in only about four percent of the affected animals (4). The occurrence was sporadic with a low morbidity and mortality and usually only a few pigs from affected litters were diseased. The outbreaks were often of short duration but in larger herds the disease could be present for many months.

The histologic lesions were similar in type to those of Teschen Disease but were milder. Both the grey and white matter were involved with the greatest intensity of lesions in the former. Perivascular cuffs consisted of cells of the lymphocytic-lymphoblastic type with a few eosinophils but rarely with neutrophils. Degenerative
changes in neurons were slight but were more common in some of the more severely affected animals. Diffuse and focal gliosis were present. The inflammation was found to be most severe in the ventral horns of the spinal cord and in the brain stem. Inflammatory changes were present in the cerebral and cerebellar cortices but were mild. A ganglionneuritis was observed in the cerebrospinal ganglia and in the abdominal sympathetic and stellate ganglia of some naturally-infected animals.

The meninges of the spinal cord, brain stem, and cerebrum were either normal or a mild non-suppurative leptomenigitis was seen. Over the cerebellum the meningitis was usually mild but in some pigs a heavy cellular infiltration was found.

The causal agent was found to be a virus, which could be transmitted to experimental pigs by intracerebral, intranasal, and oral routes of inoculation. It could be isolated from the central nervous system but not from feces, urine, or serum, of acutely affected pigs. Thordal-Christensen (76) was unable to infect white mice, guinea pigs, rabbits, cotton rats, golden hamsters, or dogs. He was also unable to establish the virus in tissue cultures prepared from testicular tissue of newborn or older pigs, or from skin, intestine, lung or spinal cord tissues of swine embryos; apparently he did not try swine kidney tissue cultures with which success was obtained with the agents of Talfan (6) and Teschen Disease (48) (58).

Aujeszky's Disease.

Heavy losses from Aujeszky's Disease have been reported in
young piglets ranging from a few days to four weeks of age (23) (51) (53) (67). The usual clinical syndrome was one with a high morbidity and mortality, and with a sudden onset, rapid prostration, neurological signs and rapid death.

The histologic lesions were those of a non-suppurative meningo-encephalitis with perivascular infiltrations, microglial proliferation and cellular infiltration in the superficial cortical zone immediately beneath the leptomeninges (34) (71). Done (10) drew attention to the constant and severe involvement of the cerebrum and cerebellum with less marked lesions in the brain stem and spinal cord; he also stated that frank necrosis could occur in the grey matter with a proportion of polymorphs in the cellular exudate. In some cases he found a number of eosinophils in the meningeal reaction. Although Hurst (34) was unable to find inclusion bodies in pigs, their presence in the neurons and neuroglia, particularly those of the cerebral cortex and less frequently in the Purkinje cells, was confirmed (10) (71).

Young pigs can be easily infected by intracerebral inoculation as well as by other routes. It is generally accepted that the rabbit is easily infected and that it is the best laboratory animal to use for laboratory diagnosis by transmission. Mice, guinea pigs, chickens and embryonated hens' eggs may also be readily infected with the virus.

Louping Ill.

This disease of sheep is not known to occur in Canada. Pigs may be infected experimentally (5) (66) and a description of the lesions
was given by Brownlee and Wilson (5). The cerebrum, cerebellum, and spinal cord showed a marked meningitis which consisted of mononuclear cells with a small proportion of eosinophils. Perivascular cuffing and focal and diffuse infiltrations were noted in the cerebrum, spinal cord and cerebellum, especially in the latter in the molecular and Purkinje cell layers; the occurrence of lesions in the brain stem was not described. The virus of Louping Ill will infect mice (5) (26) and it has been grown on the chorioallantoic membrane of embryonated hens' eggs (63).

Hog Cholera.

Numerous authors have reported on the neurologic lesions of Hog Cholera (see Done 10). Typically the disease causes a panencephalitis with a variable but usually mild myelitis. The mesodermal tissue is mainly affected and a severe vasculitis particularly of the precapillaries is constantly present. The endothelial cells undergo degeneration and proliferation and the lumens of the vessels become narrowed; thrombosis and hemorrhage into the vessel walls and perivascular tissue may occur. The vessel walls become edematous and infiltrated with round cells forming perivascular cuffs of variable width. Microglia proliferate and form glial nodules which are usually loose, but this gliosis is less conspicuous than the vascular lesions (31). Done (10) observed that the glial reaction was most evident in the neighbourhood of blood vessels, ventricles, and aqueduct.

Both the grey and white matter are involved equally (31) or with the latter containing a slightly higher intensity of lesions (10).
Authors agree that the inflammation is most severe in the brain stem with less extensive changes in the cerebral and cerebellar cortices. The leptomeninges and choroid plexuses are inflammed (10) but a distinct leptomeningitis may not be found (31).

The virus of Hog Cholera causes disease in swine only (27); pigs of all ages are susceptible unless they have developed immunity previously and, for practical purposes, intracerebral inoculation of susceptible pigs is constantly fatal.

Japanese B Encephalitis.

This disease of man occurs in the Far East (63) and is not known to occur in Canada. Ochi (62) described the disease in pigs; in sows it produced abortion and still-birth, and, in young piglets, encephalitis with lesions similar to those described in man. The microscopic picture in man is that of a non-suppurative meningitis of moderate degree, with perivascular infiltrations and nodular collections of cells in the parenchyma. These changes are scattered through the cerebral cortex, basal ganglia, brain stem and spinal cord. In the cerebellum nodules were confined to the molecular layer with some loss of Purkinje cells (25). The encephalitis in pigs is apparently similar to that of Teschen Disease. The virus can be grown in mice and eggs (63) and in tissue cultures of swine kidney (49).

Miscellaneous Non-Suppurative Encephalomyelitides of Swine.

Rabies produces a diffuse non-suppurative encephalomyelitis with inflammatory lesions in the craniospinal ganglia. Negri bodies may
be found, especially in the Purkinje cells of the cerebellum. The disease is sporadic in its occurrence and attacks pigs of all ages. 

African Swine Fever has many features in common with Hog Cholera but was found to be immunologically distinct from it. Maurer et al (56) described the pathology and they found that the lesions differed from those of Hog Cholera in minor details only.

The disease has not been found in the United States or in Canada.

Verlinde (80) described a non-suppurative encephalomyelitis which occurred in Holland in 1947. The lesions were similar to those of Teschen Disease but definite proof that it was Teschen Disease was not presented at that time.

Encephalomyelitis of piglets was described in Portugal by Tropa and Correia Madeira (78). The disease was found to be similar to Teschen Disease but differed from it in being of milder nature.

McNutt and Packer (53) isolated the virus of Western Equine Encephalomyelitis from swine and found that clinical illness could be produced in very young pigs. Karstad and Hanson (41) found that pigs were susceptible to the virus of Eastern Equine Encephalomyelitis but clinical encephalitis was not found. They described histologic lesions in one experimental animal. The lesions consisted of a perivascular infiltration with leucocytes, meningitis and "microabscesses"; these lesions were similar to those found in horses with the disease. The encephalitis in the pig was most severe in the midbrain and medulla oblongata.

A lymphocytic polioencephalomyelitis was described by Glässer (22) in pigs suffering from a subacute form of Swine Influenza. The histologic
lesions were similar to those of Teschen Disease, but further evidence that the encephalitis and respiratory disorder were related was not given.

A poliomyelitis has been reported recently in Poland (38). It had a low mortality and was transmissible by subcutaneous and oral routes of inoculation. Janowski and Zulinski (38) pointed out that the disease resembled Talfan Disease and Poliomyelitis suum of Denmark.

A virus, called the hemagglutinating virus of Japan, was found to cause disease in pigs (36) (68). It affected pigs aged from two to five months and the disease it produced was characterized clinically by nervous signs, muscular spasm, convulsions and dullness. The mortality was over 75 percent. Ishitani et al (37) described histologic changes in the central nervous system which consisted of perivascular cuffing in the frontal lobes and malacia in the midbrain and pons. Sashara et al (68) found that the virus was similar serologically to the virus isolated from the lungs of pigs with an influenza-like disease. It was believed to belong to the Mumps - Newcastle Disease - Influenza group of viruses, but did not have an antigenic component in common with the viruses of Influenza, Swine Influenza, or Newcastle Disease (36). Mice, young rabbits, adult rats, hamsters, guinea pigs (69), eggs (36), and tissue cultures of swine kidney (72) could be infected with the virus.
DISCUSSION

It is now in order to compare and contrast "Ontario Encephalomyelitis of Swine" with the diseases described above; many of the latter can be eliminated simply on epidemiological and histological grounds.

The neurologic lesions produced by the viruses of Hog Cholera and African Swine Fever are those of a primary mesodermal reaction and, in addition, the lesions are not limited to the nervous system but systemic changes occur frequently; the pathology, together with the very different epidemiologic and clinical pictures clearly distinguishes these diseases from "Ontario Encephalomyelitis". Done (10) classifies the hemagglutinating virus of Japan with those viruses primarily affecting mesodermal tissue and the lesions described by Ishitani et al (37) are distinct from those of the Ontario disease.

On a clinical basis, Aujeszky's disease would be difficult to distinguish from "Ontario Encephalomyelitis" since both diseases may cause heavy losses with nervous signs in young piglets. Aujeszky's disease produces a severe involvement of the cortices of the cerebrum and cerebellum with a less marked inflammation of the brain stem and cord. Frank necrosis also occurs in the grey matter and Done (10) confirms the observation of Shahan et al (71) that inclusion bodies occur. The distribution of lesions in "Ontario Encephalomyelitis" is the reverse of that described in Aujeszky's Disease, and in addition, inclusion bodies and frank necrosis have not been observed in over 100 cases of the Ontario disease.
Rabies, Japanese B Encephalitis and Louping Ill cause histologic lesions which could be confused with those of the Ontario disease. Neither Japanese B Encephalitis nor Louping Ill have been recognized in Canada; the former produces stillbirths and abortions in affected sows and, as far as is known, the latter does not produce natural disease in pigs. Rabies is readily differentiated on the basis of its epidemiology and the inclusion bodies it produces.

Although the viruses of the equine encephalitides appear to infect pigs readily, clinical disease is rare. In all probability, the lesions described in the central nervous system of pigs with Swine Influenza cannot be differentiated from those observed in the Ontario disease but respiratory disease was not seen in the latter and it was present in the herds described by Glässer (22); this may well have been a concurrent infection of Swine Influenza and Teschen Disease.

Teschen Disease, Talfan Disease and Poliomyelitis suum of Denmark form a group of diseases in which the character of the lesions is similar. On the basis of published illustrations and observations on sections from Teschen Disease obtained from the Armed Forces Institute of Pathology in Washington, and on sections of Talfan Disease obtained from Mr. Done of Weybridge, England, they cannot be differentiated from "Ontario Encephalomyelitis". The viruses of Teschen Disease, Talfan Disease and Poliomyelitis suum are related serologically, and Done and Harding (11) found, in a comparative study, that Talfan Disease and Teschen Disease could not be differentiated on their neuropathology. From this it appears that Talfan Disease and Poliomyelitis suum are not distinct diseases but are milder manifestations of Teschen Disease than
are classically described for it.

On a clinical basis, "Ontario Encephalomyelitis" differs from Teschen Disease, Talfan Disease and Poliomyelitis suum in affecting chiefly a younger age group of piglets, but like Teschen Disease it takes the form of an acute convulsive syndrome with a high mortality which is in contrast to the paralytic syndrome with infrequent convulsions and low morbidity and mortality seen in Talfan Disease and Poliomyelitis suum.

In all four diseases there are striking resemblances in the distribution, as well as the character, of the histologic lesions. The grey matter is inflamed more severely than the white matter, the brain stem is the site of severe changes, the lesions in the cerebrum are usually mild and variable, and there is a severe ganglionneuritis. The Ontario disease differs in having milder lesions in the spinal cord with the dorsal horns mainly affected which is in contrast to the lesions of the other diseases in which severe changes are found in the spinal cord particularly in the ventral horns. It also differs in having milder lesions in the cerebellar cortex. Classical descriptions of Teschen Disease mention a marked inflammation with prominent degeneration and glial reaction in the Purkinje cell layer. However, in the few older pigs examined the lesions were comparable in severity to those of Teschen Disease.

It should be noted that Karin Fischer (17) found a diminished intensity of lesions in the cerebellum of pigs infected naturally with the virus of Teschen Disease in contrast to the changes observed in animals infected experimentally by intracerebral and intranasal routes.
Furthermore, the cerebellar changes in Talfan Disease are milder than those described for Teschen Disease (28) and they are mild in Poliomyelitis suum (76). Perhaps the mild cerebellar lesions usually encountered in "Ontario Encephalomyelitis" can be accounted for in part by the age of the animals and the short duration of the disease. Fischer (17) in a detailed study of the pattern of development of lesions in natural cases of Teschen Disease as well as in the experimental disease induced by various routes of inoculation, has shown that the cerebellar lesions develop relatively late in the course of infection and as much as a week after extension of the virus and inflammation throughout the cerebrospinal axis. Involvement of the cerebellar cortex in Teschen Disease is by extension of destructive inflammation from the cerebellar meninges and since a mild cerebellar meningitis is almost constantly present in the Ontario disease the relative absence of cortical lesions may be explainable on the basis of the brief course of the disease in the young pigs. The relatively mild lesions in the spinal cord in "Ontario Encephalomyelitis" may also be explained on the basis of the short duration of the disease and this probability is supported by the observations of Manuelidis et al (55) who found that the changes in the spinal cord developed later than those in the brain.

A ganglionneuritis was found in the sympathetic ganglia in "Ontario Encephalomyelitis" and similar lesions have been described in Teschen Disease (55) and Poliomyelitis suum (76). In the latter disease the lesions were found only in natural infections and not in experimental
animals, and Thordal-Christensen (76) suggested that the infectious agent may possibly enter through the alimentary tract. The observations of Manuelidis et al (55) do not support this hypothesis since they found these lesions in pigs inoculated intracerebrally, intranasally and orally.

It is apparent then that apart from differences in the clinical disease and minor differences in the distribution of histologic lesions, "Ontario Encephalomyelitis" cannot be distinguished from Teschen Disease, Talfan Disease or Poliomyelitis suum. On the basis of neuropathology, Portuguese Encephalomyelitis of piglets and the non-suppurative poli-encephalomyelitis of pigs in Holland, described by Verlinde (30), are very similar to Teschen Disease also, but serologic studies are required to confirm their classification with it.

Normal figures for the total white cell count on the cerebrospinal fluid of pigs are scarce. Fankhauser (16) found normal values of $1/3$ to $20/3^*$ and sometimes counts as high as $50/3$ occurred normally. He does not give the age of the pigs examined. The figures reported in this thesis on a small number of normal piglets are higher and a mean value of $48.2$ cells/cm³ with a maximum of $74$ and a minimum of $29$ cells/cm³ was obtained. This disparity of the normal figures may be due to the age of the pigs examined since here piglets under eight days of age were used and if human figures can be taken as a guide, normal figures are considerably higher in the very young (24).

*Fankhauser does not give an explanation of the expression "$/3^*$ but is interpreted to mean "per cm³".
Fankhauser (16) examined the cerebrospinal fluid from animals infected with Teschen Disease and found counts of 48/3 to 209/3. Fischer and Starke (18) observed counts varying from 14/3 to 3004/3 with an average of 517/3 in pigs with Teschen Disease. They reported that a rise in mononuclear cells occurred mainly during the beginning of the paralytic stage and decreased thereafter. Polymorphs were not found and this is in contrast to the changes observed in Poliomyelitis of humans with which Teschen Disease is often compared. The figures found in the Ontario disease varied from 33 to 583 cells/cmm and these are either normal or mildly increased in comparison to the figures found in normal pigs. These figures are comparable to those reported in Teschen Disease but the elevation of white cells is not so striking. The Pandy test revealed the presence of excess protein and this result is in accordance with findings in Teschen Disease (16) (18).
OBSERVATIONS ON TRANSMISSIBILITY OF "ONTARIO ENCEPHALOMYELITIS OF SWINE"

Epidemiologic and histologic observations indicated that the disease being studied was infectious and that the causative agent was probably a virus. The following experiments on transmissibility were undertaken to test these possibilities and pigs, laboratory animals, embryonated hens' eggs and tissue cultures were used as the test systems.

Materials and Methods

Brain material from natural cases of "Ontario Encephalomyelitis" was used in the inoculum. Half-brains were collected from naturally-infected pigs which were killed immediately prior to autopsy. The material was collected within 30 minutes of death and stored at either -15°C or -40°C until required for use (see Table II). In each case a histologic examination was made on the other part of the brain to confirm the diagnosis. Material from 10 different field outbreaks (numbered 1 to 10) was used; sometimes the samples were pooled but usually material from individual outbreaks was tested alone.

The inoculum was prepared in a TenBroeck grinder as a 10 or 20 percent suspension of brain material in tryptose-phosphate broth or physiological saline which was then centrifuged at 1500 rpm for 10 minutes. The supernate was treated with antibiotic or filtered through an ST1 Seitz filter. Antibiotic was usually added at the concentrations of either 100 IU procaine penicillin and 10 mg dihydrostreptomycin per 1 ml or twice this rate; both concentrations were satisfactory for controlling bacterial
### TABLE II

Details of Preservation of Brain Material Used for Transmission

<table>
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<th>Material*</th>
<th>Used in Expt. No.</th>
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<th>Total days before use</th>
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<td>70</td>
<td>XI</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>+</td>
</tr>
</tbody>
</table>

* = Material from field outbreaks nos. 1, 2 and 3 was pooled; material from pigs nos. 64, 65 and 66 was pooled; and material from pigs nos. 69 and 70 was pooled.

** = First in a deep freeze at -15°C and then transferred to a dry ice chest at -40°C.

/ = Results of transmission tests with pigs.
contaminants. In some experiments the supernate was used untreated, and frequently the inoculum was not centrifuged but antibiotic was added.

An antibiotic-treated supernate of a 10 percent suspension of brain was the usual inoculum for laboratory animals and embryonated hens' eggs and the techniques of inoculation were those that are usually employed in such investigations (42).

The inoculum for controls was prepared from brain material of normal suckling piglets by the same methods as those used for the infective material.

Figs

The animals used in the transmission experiments were from four hours to 24 days of age and were obtained locally from two piggeries of Yorkshire, Landrace, and crossbred pigs. Two groups of pigs were used - those which had received colostrum before they were placed on an artificial diet, and those which were colostrum-free. All piglets which had received colostrum had their daily temperatures recorded from the time of inoculation until they died or were killed. Similar records were not kept on colostrum-free pigs since it was desirable to handle the piglets as little as possible to keep bacterial contamination of the environment low. Each colostrum-free pig was kept in a separate isolation unit whereas pigs which received colostrum were kept as groups in isolation rooms.

Intracerebral, intranasal, and oral routes of inoculation were tested. Intracerebral inoculation was usually done under ether anesthesia, but in some of the younger pigs no anesthetic was used. The inoculum was
injected through a trephine opening over the left cerebral hemisphere. A syringe and needle was used for intranasal inoculation and oral administration was done by placing the inoculum in the milk and/or introducing it into the pharynx with a syringe and needle.

The spleen, jejunum, and brain, were cultured for bacteria at autopsy of each pig, except that in those cases which received colostrum the brain was not cultured. The results of these bacteriologic findings will not be considered further except where they are thought to be of some significance, in which case they are mentioned in the results of individual experiments.

Whenever possible experimental animals were killed and not left to die naturally; they were killed when they showed clinical signs of disease or at arbitrary times after inoculation if they did not sicken. Brain material was saved from selected pigs for serial passages. The sole criterion of a positive transmission was the finding of typical lesions of "Ontario Encephalomyelitis" in histologic sections which were prepared from at least five levels of the brain of each experimental pig. When lesions were present they were classified on their intensity as one plus when they were mild or two and three plus when they were of a severity similar to that found in naturally infected pigs. Pigs with suspicious changes were classified as negative.

Details of the methods and results of the individual experiments are given in Tables III to XIII.
Experiment I. (See Table III)

Pigs which had received colostrum were used; they were between two and three weeks old at the time of inoculation. Eleven piglets (nos. 1 to 11) were inoculated intracerebrally with either a Seitz-filtered or antibiotic-treated inoculum prepared from a pooled sample of three field cases (field outbreaks nos. 1, 2, and 3).

Results. Pig no. 11 died on the day of inoculation from trauma. Pig no. 2 which received the antibiotic-treated material, vomited and was ataxic 11 days after inoculation; it became weaker and was killed on the thirteenth day. Some of the other pigs became slightly ataxic but this was of a very transient nature and clinically it was difficult to ascertain that they were diseased. The pigs were killed on the fourteenth or nineteenth days after inoculation.

Pig no. 2 showed typical lesions of a severity similar to those seen in naturally-infected pigs. Three other pigs (nos. 1, 3, and 7) also showed lesions but in these they were mild. Pig no. 7 had been inoculated with a Seitz filtrate; the other pigs with lesions had received the antibiotic-treated inoculum.

Experiment II. (See Table IV)

The purpose of this experiment was to attempt a second passage with material from Experiment I.

Piglets, which had received colostrum, were used; they were 12 to 15 days old at the time of inoculation. Part of the brain from pig no. 2 was prepared as antibiotic-treated and as Seitz-filtered suspensions and it was inoculated into nine pigs (nos. 12 to 20)
# TABLE III

Results of Transmission Experiment I
Using Pigs which Received Colostrum.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Material</th>
<th>Inoculum Treatment</th>
<th>Route and Dose</th>
<th>Age at Inocul. (weeks)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*</td>
<td>antib.</td>
<td>i/o, 0.2 ml</td>
<td>3</td>
<td>19k</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>*</td>
<td></td>
<td></td>
<td>3</td>
<td>13k</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>*</td>
<td></td>
<td></td>
<td>2-3</td>
<td>14k</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>*</td>
<td></td>
<td></td>
<td>2-3</td>
<td>14k</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>*</td>
<td></td>
<td></td>
<td>2-3</td>
<td>19k</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>*</td>
<td></td>
<td></td>
<td>2-3</td>
<td>19k</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>*</td>
<td>Seitz</td>
<td></td>
<td>2-3</td>
<td>14k</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>*</td>
<td></td>
<td></td>
<td>3</td>
<td>14k</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>*</td>
<td></td>
<td></td>
<td>3</td>
<td>19k</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>*</td>
<td></td>
<td></td>
<td>3</td>
<td>14k</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>*</td>
<td></td>
<td></td>
<td>2-3</td>
<td>ln /</td>
<td>-</td>
</tr>
</tbody>
</table>

* = pool of brain material from field outbreaks nos. 1, 2, and 3; supernate of 10% suspension.

i/o = intracerebral.
k = killed.
n = died.
/ = unrelated disease.
- = not done.
### TABLE IV

Results of Transmission Experiment II Using Pigs which Received Colostrum.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Inoculum Material</th>
<th>Treatment</th>
<th>Route and Dose</th>
<th>Age at Inocul. (weeks)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>*</td>
<td>antib.</td>
<td>i/o, 0.2 ml</td>
<td>2</td>
<td>5k</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2</td>
<td>6k /</td>
<td>o</td>
</tr>
<tr>
<td>14</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2</td>
<td>5k /</td>
<td>o</td>
</tr>
<tr>
<td>15</td>
<td>*</td>
<td>&quot;</td>
<td>i/n, 0.5 ml</td>
<td>2</td>
<td>4n /</td>
<td>o</td>
</tr>
<tr>
<td>16</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2</td>
<td>6k</td>
<td>o</td>
</tr>
<tr>
<td>17</td>
<td>*</td>
<td>Seitz</td>
<td>i/o, 0.2 ml</td>
<td>2</td>
<td>15k</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2</td>
<td>15k</td>
<td>o</td>
</tr>
<tr>
<td>19</td>
<td>*</td>
<td>&quot;</td>
<td>i/n, 0.5 ml</td>
<td>2</td>
<td>15k</td>
<td>o</td>
</tr>
<tr>
<td>20</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2</td>
<td>15k</td>
<td>o</td>
</tr>
<tr>
<td>21</td>
<td>**</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>15k</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>**</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>15k</td>
<td>o</td>
</tr>
</tbody>
</table>

* = brain from Pig no. 2, from Experiment I; supernate 10% suspension.
** = contact control.
i/o = intracerebral.
i/n = intranasal.
k = killed.
n = died.
/ = unrelated disease, see text.
- = not done.
intracerebrally or intranasally. Two contact controls were added to the group.

Results. Three pigs (nos. 13, 14, and 15) developed a coliform enteritis and they were removed from the pen. The remainder did not show clinical illness and were killed at various times from five to 15 days after inoculation. Mild lesions of a non-suppurative encephalitis were found in three pigs (nos. 12, 17, and 21); pig no. 12 was inoculated with antibiotic-treated material, pig no. 17 with the Seitz filtrate, and pig no. 21 was a contact control. None of the pigs dosed intranasally showed lesions of encephalitis.

Experiment III. (See Table V)

In an attempt to increase the success of transmission, material from another field outbreak and cerebrospinal fluid from the sick pigs was used in the inoculum. At this time one of the piggeries from which some of the pigs for these transmission experiments had been obtained, experienced a natural outbreak of this disease and material from this outbreak (no. 5) was used. The brain material was prepared as a 10 percent suspension in a mixture of cerebrospinal fluid and tryptose-phosphate broth. Thirteen pigs (nos. 23 to 35) were used in the experiment, and of these, four were given a Seitz filtrate, five were given the suspension with antibiotics, two were used as contact controls, and two were controls which were inoculated with a normal brain suspension and kept in an isolation room separate from the other pigs. The inoculated pigs were given the inoculum by the intracerebral route. They had received colostrum and they were 10 or 17 days old at the time of inoculation.
<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Material</th>
<th>Inoculum Treatment</th>
<th>Route and Dose</th>
<th>Age at Inocul. (days)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>*</td>
<td>antib.</td>
<td>1/c, 0.5 ml</td>
<td>17</td>
<td>3n /</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>17</td>
<td>38k</td>
<td>o</td>
</tr>
<tr>
<td>25</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>25k</td>
<td>o</td>
</tr>
<tr>
<td>26</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>44k</td>
<td>o</td>
</tr>
<tr>
<td>27</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>25k</td>
<td>o</td>
</tr>
<tr>
<td>28</td>
<td>*</td>
<td>Seitz</td>
<td>&quot;</td>
<td>10</td>
<td>25k</td>
<td>o</td>
</tr>
<tr>
<td>29</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>17</td>
<td>44k</td>
<td>o</td>
</tr>
<tr>
<td>30</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>17</td>
<td>25k</td>
<td>o</td>
</tr>
<tr>
<td>31</td>
<td>*</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>38k</td>
<td>o</td>
</tr>
<tr>
<td>32</td>
<td>**</td>
<td>antib.</td>
<td>&quot;</td>
<td>17</td>
<td>44k</td>
<td>o</td>
</tr>
<tr>
<td>33</td>
<td>**</td>
<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>24k</td>
<td>o</td>
</tr>
<tr>
<td>34</td>
<td>***</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>48k</td>
<td>o</td>
</tr>
<tr>
<td>35</td>
<td>***</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>25k</td>
<td>o</td>
</tr>
</tbody>
</table>

* = brain material and cerebrospinal fluid from field outbreak no. 5; supernate of 10% suspension.
** = normal brain; supernate of 10% suspension.
*** = contact control.
1/c = intracerebral.
n = died.
k = killed.
= not done.
= unrelated disease, see text.
Results. Pig no. 23 died three days after inoculation and it was found to have an imperforate anus. The other pigs remained clinically normal and were killed 25 to 44 days after inoculation. Histological lesions were not found in the brains of these animals.

Experiment IV. (See Table VI)

It was decided to test inocula without antibiotic or Seitz filtration, and to see if cortisone would increase the susceptibility of the pigs.

Six pigs (nos. 36 to 41), which had received colostrum, were inoculated intracerebrally with a suspension prepared from either a pool from three field outbreaks (nos. 1, 2, and 3), or material from field outbreak no. 6. The pigs were either two or three weeks old. Three pigs were given the inoculum untreated and, in an attempt to increase their susceptibility, the other pigs (nos. 36, 37, and 40) were given 200 mg of cortisone acetate* intramuscularly in two doses, the first, two days before and the second three hours before inoculation with the same brain suspension treated with antibiotics. One animal from each of these two groups received the inoculum prepared from field outbreak no. 6, and the pooled sample was used in the others.

Results. Two of the pigs (nos. 38 and 39) that received the untreated suspension developed a coliform purulent meningitis. Clinical signs were not seen in the other pigs and when autopsied 22 days after inoculation, very mild histologic lesions of encephalitis were found in

*Saline suspension of Cortone Acetate (cortisone acetate, Merck).
TABLE VI

Results of Transmission Experiment IV
Using Pigs which Received Colostrum.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Material</th>
<th>Treatment</th>
<th>Route and Dose</th>
<th>Age at Inocul. (weeks)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>36'</td>
<td>*</td>
<td>antib.</td>
<td>i/c, 0.5 ml</td>
<td>3</td>
<td>22k</td>
<td>+</td>
</tr>
<tr>
<td>37'</td>
<td>*</td>
<td></td>
<td>&quot;</td>
<td>3</td>
<td>22k</td>
<td>+</td>
</tr>
<tr>
<td>38</td>
<td>*</td>
<td>none</td>
<td>&quot;</td>
<td>2</td>
<td>3k /</td>
<td>o</td>
</tr>
<tr>
<td>39</td>
<td>*</td>
<td></td>
<td>&quot;</td>
<td>2</td>
<td>11k /</td>
<td>o</td>
</tr>
<tr>
<td>40'</td>
<td>**</td>
<td>antib.</td>
<td>&quot;</td>
<td>3</td>
<td>22k</td>
<td>o</td>
</tr>
<tr>
<td>41</td>
<td>**</td>
<td>none</td>
<td>&quot;</td>
<td>2</td>
<td>22k</td>
<td>o</td>
</tr>
</tbody>
</table>

* = pool of brain material from field outbreaks nos. 1, 2, and 3; supernate of 10% suspension.
** = brain from field outbreak no. 6; supernate of 10% suspension.
i/c = intracerebral.
k = killed.
/ = unrelated disease, see text.
+ = received 200 mg of cortisone acetate intramuscularly in two doses, the first two days before and the second three hours before inoculation.
two pigs (nos. 37 and 36) which had received cortisone and the pooled sample. The cortisone did not appear to influence greatly the success of transmission in this limited trial.

Experiment V. (See Table VII)

In this experiment the dose of inoculum was increased by giving it both intracerebrally and intranasally, and, in addition, material from two other field outbreaks (nos. 7 and 8) was tested for infectivity. The inocula were prepared as 10 percent suspensions and each was used untreated in one pig and antibiotic-treated in two. The six pigs (nos. 42 to 47) were each inoculated by intracerebral and intranasal routes; they had received colostrum and they were one week old when inoculated.

Results. No clinical signs were seen other than a mild diarrhoea in two pigs (nos. 43 and 45) which resolved itself spontaneously. The pigs were killed 17 days after inoculation. The histological examination of the brains failed to reveal lesions of encephalitis in any of the pigs.

Experiment VI. (See Table VIII)

Material from two more field outbreaks (nos. 4 and 9) were tested by other routes of inoculation. Spleen and lymph node were used in the second of these inocula in addition to brain. Furthermore, it was decided to use uncentrifuged 10 percent suspensions treated with antibiotic, in contrast to those of the previous experiments where only centrifuged suspensions had been used. Six three-week-old pigs (nos. 48 to 53)
TABLE VII
Results of Transmission Experiment V
Using Pigs which Received Colostrum.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Material</th>
<th>Inoculum</th>
<th>Route and Dose</th>
<th>Age at Inocul. (weeks)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>*</td>
<td>antib.</td>
<td>#</td>
<td>1</td>
<td>17k</td>
<td>o</td>
</tr>
<tr>
<td>43</td>
<td>*</td>
<td>&quot;</td>
<td>#</td>
<td>1</td>
<td>17k</td>
<td>o</td>
</tr>
<tr>
<td>44</td>
<td>*</td>
<td>none</td>
<td>#</td>
<td>1</td>
<td>17k</td>
<td>o</td>
</tr>
<tr>
<td>45</td>
<td>**</td>
<td>antib.</td>
<td>#</td>
<td>1</td>
<td>17k</td>
<td>o</td>
</tr>
<tr>
<td>46</td>
<td>**</td>
<td>&quot;</td>
<td>#</td>
<td>1</td>
<td>17k</td>
<td>o</td>
</tr>
<tr>
<td>47</td>
<td>**</td>
<td>none</td>
<td>#</td>
<td>1</td>
<td>17k</td>
<td>o</td>
</tr>
</tbody>
</table>

* = brain from field outbreak no. 7; supernate of 10% suspension.
** = brain from field outbreak no. 8; supernate of 10% suspension.
# = intracerebrally, 0.5 ml and intranasally, 0.5 ml.
k = killed.
### TABLE VIII

Results of Transmission Experiment VI
Using Pigs which Received Colostrum.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Material Treatment</th>
<th>Inoculum Route and Dose</th>
<th>Age at Inocul. (weeks)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>48'</td>
<td>* antib.</td>
<td>i/c, 1.0 ml</td>
<td>3</td>
<td>23k</td>
<td>o</td>
</tr>
<tr>
<td>49</td>
<td>*</td>
<td>p/o, 4.0 ml</td>
<td>3</td>
<td>23k</td>
<td>o</td>
</tr>
<tr>
<td>50</td>
<td>*</td>
<td>s/c, 2.0 ml</td>
<td>3</td>
<td>23k</td>
<td>o</td>
</tr>
<tr>
<td>51'</td>
<td>**</td>
<td>i/c, 1.0 ml</td>
<td>3</td>
<td>23k</td>
<td>o</td>
</tr>
<tr>
<td>52</td>
<td>**</td>
<td>p/o, 4.0 ml</td>
<td>3</td>
<td>23k</td>
<td>o</td>
</tr>
<tr>
<td>53</td>
<td>**</td>
<td>s/c, 2.0 ml</td>
<td>3</td>
<td>23k</td>
<td>o</td>
</tr>
</tbody>
</table>

* = brain from field outbreak no. 4; 10% suspension.
** = brain, spleen, and lymph nodes from field outbreak no. 9; 10% suspension.
i/c = intracerebral.
p/o = oral.
s/c = subcutaneous.
k = killed.
i' = received 125 mg of cortisone acetate intramuscularly four hours before inoculation.
which had received colostrum, were used and each inoculum was tested in a pig by the intracerebral, subcutaneous, or oral routes. The intracerebrally-inoculated pigs also received 125 mg of cortisone acetate intramuscularly four hours before inoculation.

Results. These pigs remained healthy and they were killed 23 days after inoculation. There were no lesions of encephalitis in them.

Experiment VII. (See Table IX)

Due to the relatively poor results obtained so far and to the likelihood that the piglets were receiving a degree of immunity from the colostrum, colostrum-free piglets were used in this experiment. Two inocula were used as supernates of 10 percent suspensions; one was prepared from field outbreak no. 7 while the other contained a pool of material from field outbreaks nos. 1, 2, and 3. Eight colostrum-free piglets (nos. 54 to 61) were used when they were four days old. Each inoculum was used with antibiotic in two pigs, and one pig received a Swinney-filtered preparation; two control pigs were given a normal brain suspension and one control was left uninoculated. All inoculations were by the intracerebral route.

Results. The pig (no. 57) given Swinney-filtered material developed a streptococcal meningitis. Specific clinical signs were not seen in the other pigs and they were killed from 14 to 46 days after inoculation. Only one pig, pig no. 59, which was killed 23 days after inoculation, had lesions of encephalitis, and these were very mild. It had been inoculated with material that had been frozen for a minimum
TABLE IX

Results of Transmission Experiment VII
Using Colostrum-Free Pigs.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Material</th>
<th>Treatment</th>
<th>Route and Dose</th>
<th>Age at Inocul. (days)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>18k</td>
<td>o</td>
</tr>
<tr>
<td>55</td>
<td>**</td>
<td>antib.</td>
<td>1/c, 0.5 ml</td>
<td>4</td>
<td>46k</td>
<td>o</td>
</tr>
<tr>
<td>56</td>
<td>**</td>
<td>&quot;</td>
<td>&quot;</td>
<td>4</td>
<td>14k</td>
<td>o</td>
</tr>
<tr>
<td>57</td>
<td>***</td>
<td>Swinney</td>
<td>&quot;</td>
<td>4</td>
<td>2k /</td>
<td>o</td>
</tr>
<tr>
<td>58</td>
<td>***</td>
<td>antib.</td>
<td>&quot;</td>
<td>4</td>
<td>14k</td>
<td>o</td>
</tr>
<tr>
<td>59</td>
<td>***</td>
<td>&quot;</td>
<td>&quot;</td>
<td>4</td>
<td>23k</td>
<td>+</td>
</tr>
<tr>
<td>60</td>
<td>****</td>
<td>&quot;</td>
<td>&quot;</td>
<td>4</td>
<td>46k</td>
<td>o</td>
</tr>
<tr>
<td>61</td>
<td>****</td>
<td>&quot;</td>
<td>&quot;</td>
<td>4</td>
<td>14k</td>
<td>o</td>
</tr>
</tbody>
</table>

* = uninoculated control.
** = normal brain; supernate of 10% suspension.
*** = pool of brain from field outbreaks nos. 1, 2, and 3; supernate of 10% suspension.
**** = brain from field outbreak no. 7; supernate of 10% suspension.
1/c = intracerebral.
k = killed.
/ = unrelated disease, see text.
- = not done.
period of 294 days.

Experiment VIII. (See Table X)

In the hope of obtaining more positive results, the dose rate of infective material was increased by using an uncentrifuged suspension, by using a 20 percent suspension instead of the 10 percent suspension used in the previous experiments, and by loading each piglet with inoculum by intracerebral, intranasal, and oral routes. The inoculum contained brain material from another field outbreak (no. 10) which had occurred four days before the experiment was started. Five colostrum-free pigs (nos. 62 to 66) were four hours old when three were given the infective material treated with antibiotic and two were given a control inoculum containing normal brain only.

Results. The three piglets (nos. 64, 65, and 66) given the infective material became depressed, weak, and recumbent, and they were killed on either the fourth or fifth days after inoculation. Mild but quite diagnostic lesions of "Ontario Encephalomyelitis" were found in their brains. The control pigs were normal.

Experiment IX. (See Table XI)

The main aim of this trial was to produce a second serial passage and the same methods as those of Experiment VIII were used, but infection was also attempted with Seitz-filtered material and by the oral route of inoculation alone. A 20 percent suspension was prepared from a pool of brains from pigs nos. 64, 65, and 66 from the previous experiment,
TABLE X

Results of Transmission Experiment VIII
Using Colostrum-Free Pigs.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Inoculum Material</th>
<th>Treatment</th>
<th>Route and Dose</th>
<th>Age at Inocul. (hours)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>* antib.</td>
<td>#</td>
<td></td>
<td>4</td>
<td>4k</td>
<td>0</td>
</tr>
<tr>
<td>63</td>
<td>*</td>
<td>#</td>
<td></td>
<td>4</td>
<td>10k</td>
<td>0</td>
</tr>
<tr>
<td>64</td>
<td>**</td>
<td>#</td>
<td></td>
<td>4</td>
<td>4k</td>
<td>++</td>
</tr>
<tr>
<td>65</td>
<td>**</td>
<td>#</td>
<td></td>
<td>4</td>
<td>5k</td>
<td>++</td>
</tr>
<tr>
<td>66</td>
<td>**</td>
<td>#</td>
<td></td>
<td>4</td>
<td>4k</td>
<td>++</td>
</tr>
</tbody>
</table>

* = normal brain; 20% suspension.
** = brain from field outbreak no. 10; 20% suspension.
# = intracerebral, 1.0 ml; intranasal, 0.25 ml; oral, 0.75 ml; and 1.0 ml in first feed.
k = killed.
TABLE XI
Results of Transmission Experiment IX
Using Colostrum-Free Pigs.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Inoculum Material</th>
<th>Treatment</th>
<th>Route and Dose</th>
<th>Age at Inocul. (hours)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>*</td>
<td>Seitz</td>
<td>###</td>
<td>4</td>
<td>5½n †</td>
<td>+</td>
</tr>
<tr>
<td>68</td>
<td>*</td>
<td>&quot;</td>
<td>###</td>
<td>4</td>
<td>4½k</td>
<td>++</td>
</tr>
<tr>
<td>69</td>
<td>*</td>
<td>antib.</td>
<td>#</td>
<td>4</td>
<td>6½k</td>
<td>+++</td>
</tr>
<tr>
<td>70</td>
<td>*</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>6½k</td>
<td>++</td>
</tr>
<tr>
<td>71</td>
<td>*</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>3k</td>
<td>++</td>
</tr>
<tr>
<td>72</td>
<td>*</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>3k</td>
<td>+</td>
</tr>
<tr>
<td>73</td>
<td>*</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>3k</td>
<td>+</td>
</tr>
<tr>
<td>74</td>
<td>*</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>2½k †</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>**</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>6½k</td>
<td>0</td>
</tr>
<tr>
<td>76</td>
<td>**</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>5½k</td>
<td>0</td>
</tr>
<tr>
<td>77</td>
<td>**</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>3k</td>
<td>0</td>
</tr>
<tr>
<td>78</td>
<td>**</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>14k</td>
<td>0</td>
</tr>
<tr>
<td>79</td>
<td>**</td>
<td>&quot;</td>
<td>#</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>80</td>
<td>***</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>6½k †</td>
<td>0</td>
</tr>
<tr>
<td>81</td>
<td>***</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>2½n †</td>
<td>0</td>
</tr>
</tbody>
</table>

* = pool of brains from Pigs nos. 64, 65, and 66, from Experiment VIII; 20% suspension.
** = normal brain; 20% suspension.
*** = uninoculated control.
# = intracerebral, 1.0 ml; intranasal, 0.25 ml; oral, 0.75 ml; and 1.0 ml in the first feed.
## = oral, 2.0 ml; and 1.0 ml in the first feed.
### = intracerebral, 1.5 ml; intranasal, 0.25 ml; oral, 0.75 ml; and 1.0 ml in the first feed.
k = killed.
n = died.
† = unrelated disease, see text.
- = not done.
and part of it was Seitz-filtered while the remainder was treated with antibiotic. Fifteen colostrum-free pigs (nos. 67 to 81) were used when they were four hours old in the following ways:—each of four was inoculated by intracerebral, intranasal, and oral routes with antibiotic-treated material; each of two was dosed by similar routes with the Seitz filtrate; two were infected orally; two were left as uninoculated controls; one was given a normal suspension orally; and each of four was given the normal brain suspension by the intracerebral, intranasal, and oral methods.

Results. The eight pigs inoculated with infective material became depressed, some of them vomited, and they rapidly became comatose. They were killed at various times from three to six and one-half days after inoculation.

Histological lesions of encephalitis were found in the brains of seven; the two pigs given Seitz filtrate were positive but one of them (pig no. 67) had extensive edema of the gastric submucosa and a heavy growth of hemolytic Escherichia coli was cultured from the small intestine, so, in all probability, it died from Gut Edema. Of the other six infected pigs, typical lesions were found in five but generally the lesions of encephalitis were mild and were about as severe as those found in mild natural cases; however, the lesions were more severe in the two orally-infected animals.

Pig no. 74 and the two uninoculated controls, nos. 80 and 81, developed a coliform septicemia. The other control pigs were killed at various times, which generally corresponded to the times when infected pigs were killed, and their brains were normal.
Experiment X. (See Table XII)

Again, the main aim in this experiment was to continue the serial passage of the infective agents and a third passage was made. However, since the histologic lesions in the brains of successfully infected animals were mild in comparison to those of many natural cases and since the best lesions in Experiment IX were found in the orally-infected pigs which lived longest, an attempt was made to improve the intensity of the lesions by reducing the dose of inoculum to perhaps give the changes more time to develop.

Towards these ends, 10 and 20 percent suspensions of brain were prepared from pigs nos. 69 and 70 of the previous experiment and they were given to pigs by intracerebral, intranasal, and oral routes. Six colostrum-free pigs (nos. 82 to 87) were used when they were four hours old and two received the 20 percent suspension, two the 10 percent suspension, and two controls were given a 20 percent suspension of normal brain.

Results. The infected pigs sickened and three were killed three to four days after inoculation. The fourth pig (no. 83) died naturally from a coliform septicemia. Successful transmissions were obtained in pigs nos. 82 and 84; one was given the 10 percent suspension and one the 20 percent suspension, but in both the histologic lesions were mild and the size of the dose of infective material appeared to have little effect on the intensity of the lesions.

Pig no. 85 sickened with the other pigs but its brain was normal and a bacterial septicemia was not found; the cause of death was not determined. The control pigs were normal.
TABLE XII

Results of Transmission Experiment X Using Colostrum-Free Pigs.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Inoculum Material</th>
<th>Treatment</th>
<th>Route and Dose</th>
<th>Age at Inocul. (hours)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>82</td>
<td>*</td>
<td>antib.</td>
<td>#</td>
<td>4</td>
<td>3k</td>
<td>+</td>
</tr>
<tr>
<td>83</td>
<td>*</td>
<td></td>
<td>#</td>
<td>4</td>
<td>4½n f</td>
<td>o</td>
</tr>
<tr>
<td>84</td>
<td>**</td>
<td></td>
<td>#</td>
<td>4</td>
<td>4k</td>
<td>+</td>
</tr>
<tr>
<td>85</td>
<td>**</td>
<td></td>
<td>#</td>
<td>4</td>
<td>3k f</td>
<td>o</td>
</tr>
<tr>
<td>86</td>
<td>***</td>
<td></td>
<td>#</td>
<td>4</td>
<td>4k</td>
<td>o</td>
</tr>
<tr>
<td>87</td>
<td>***</td>
<td></td>
<td>#</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* = pool of brains from Pigs nos. 69 and 70 from Experiment IX; 10% suspension.
** = pool of brains from pigs nos. 69 and 70 from Experiment IX; 20% suspension.
*** = normal brain; 20% suspension.
# = intracerebral, 1.0 ml; intranasal, 0.25 ml; oral, 0.75 ml; and 1.0 ml in the first feed.
k = killed
n = died.
f = unrelated disease, see text.
- = not done.
Experiment XI. (See Table XIII)

The third serial passage was repeated but this time only the intracerebral or oral routes alone were tried. An attempt was also made to produce a second serial passage with Seitz-filtered material.

Brain material from pig no. 68, which had been infected with a Seitz filtrate in Experiment IX and was found to have lesions of encephalitis, was prepared as a Seitz filtrate of a 20 percent suspension, and it was given intracerebrally to two pigs and orally to two others. A 20 percent suspension similar to that used in Experiment X was inoculated into two pigs intracerebrally and two pigs orally. Two control pigs were given a 20 percent suspension of normal brain orally.

Results. The pigs given Seitz filtrate were killed three and one-half to 28 days after inoculation and lesions were not found in their brains. The other four piglets given unfiltered material sickened and were autopsied three and one-half to seven days after inoculation. Pigs nos. 92 and 93, which were inoculated intracerebrally, and pig no. 95, which was inoculated orally, were positive but lesions were not found in pig no. 94 from which pathogenic bacteria were not cultured and the cause of its sickness was not determined. One of the control pigs (no. 96) developed hepatitis and arthritis caused by Pseudomonas spp. while the other control was normal.

Review of Transmission Experiments with Pigs

Brain material from 10 field outbreaks of "Ontario Encephalomyelitis" has been used to test the transmissibility of the disease in
TABLE XIII
Results of Transmission Experiment XI
Using Colostrum-Free Pigs.

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Inoculum Material</th>
<th>Inoculum Treatment</th>
<th>Route and Dose</th>
<th>Age at Inocul. (hours)</th>
<th>Days to Death</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>88</td>
<td>*</td>
<td>Seitz</td>
<td>p/o, 2.0 ml</td>
<td>5</td>
<td>23k</td>
<td>0</td>
</tr>
<tr>
<td>89</td>
<td>*</td>
<td></td>
<td></td>
<td>5</td>
<td>3.5k</td>
<td>0</td>
</tr>
<tr>
<td>90</td>
<td>*</td>
<td></td>
<td>i/c, 1.5 ml</td>
<td>5</td>
<td>7k</td>
<td>0</td>
</tr>
<tr>
<td>91</td>
<td>*</td>
<td></td>
<td></td>
<td>5</td>
<td>28k</td>
<td>0</td>
</tr>
<tr>
<td>92</td>
<td>**</td>
<td>antib.</td>
<td>i/c, 1.0 ml</td>
<td>5</td>
<td>3.5k</td>
<td>++</td>
</tr>
<tr>
<td>93</td>
<td>**</td>
<td></td>
<td></td>
<td>5</td>
<td>3.5k</td>
<td>+</td>
</tr>
<tr>
<td>94</td>
<td>**</td>
<td></td>
<td></td>
<td>5</td>
<td>5k /</td>
<td>0</td>
</tr>
<tr>
<td>95</td>
<td>**</td>
<td></td>
<td></td>
<td>5</td>
<td>7k</td>
<td>+</td>
</tr>
<tr>
<td>96</td>
<td>***</td>
<td></td>
<td>p/o, 3.0 ml</td>
<td>5</td>
<td>14k /</td>
<td>0</td>
</tr>
<tr>
<td>97</td>
<td>***</td>
<td></td>
<td></td>
<td>5</td>
<td>7k</td>
<td>0</td>
</tr>
</tbody>
</table>

* = brain from Pig no. 68 of Experiment IX; supernate of 20% suspension.
** = pool of brains from Pigs nos. 69 and 70 of Experiment IX; 20% suspension.
*** = normal brain; 20% suspension.
# = oral, 2.0 ml; and 1.0 ml in the first feed.
i/c = intracerebral.
p/o = oral.
k = killed.
/ = unrelated disease, see text.
<table>
<thead>
<tr>
<th>Material</th>
<th>Treatment of inoculum</th>
<th>Route of inoculation</th>
<th>No. of pigs</th>
<th>No. with lesions</th>
<th>No. of unrelated deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infective brain</td>
<td>antibiotic</td>
<td>i/c</td>
<td>19</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>i/c,i/n</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>i/n</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>p/o</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>s/c</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>Seitz filt.</td>
<td>i/c</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>i/n</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>untreated</td>
<td>i/c</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>i/c,i/n</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL EXPERIMENTAL</td>
<td></td>
<td></td>
<td>47</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Contact controls</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Normal brain</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL CONTROLS</td>
<td></td>
<td></td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

i/c = intracerebral.
i/n = intranasal.
p/o = oral.
s/c = subcutaneous.
### TABLE XV

**Summary of Results of Transmission Experiments with Colostrum-Free Pigs.**

<table>
<thead>
<tr>
<th>Material</th>
<th>Treatment of inoculum</th>
<th>Route of inoculation</th>
<th>No. of pigs</th>
<th>No. with lesions</th>
<th>No. of unrelated deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infective brain</td>
<td>antibiotic</td>
<td>i/c; i/n, p/o</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>i/c; i/n, p/o</td>
<td>11</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>p/o</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>Seitz filt.</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>i/c; i/n, p/o</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>p/o</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>Swinney filt.</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL EXPERIMENTAL</strong></td>
<td></td>
<td></td>
<td>28</td>
<td>16</td>
<td>5</td>
</tr>
</tbody>
</table>

| Normal brain      | antibiotic            | i/c; i/n, p/o        | 2           | 0                | 0                      |
|                   | "                     | i/c; i/n, p/o        | 8           | 0*               | 0                      |
|                   | "                     | p/o                  | 3           | 0*               | 1                      |
| **Uninoculated controls** |                 |                      | 3           | 0                | 2                      |
| **TOTAL CONTROLS** |                       |                      | 16          | 0                | 3                      |

*i/c = intracerebral.  
i/n = intranasal.  
p/o = oral.  
* = includes one pig not killed.
# TABLE XVI

General Summary of Results of Transmission Experiments with Pigs.

<table>
<thead>
<tr>
<th>Group of pigs</th>
<th>No. of pigs used</th>
<th>No. with lesions</th>
<th>No. of unrelated deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs given untreated infective brain</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Pigs given antibiotic-treated infective brain</td>
<td>50</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Pigs given filtered infective brain</td>
<td>20</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>Suckled pigs given infective brain</td>
<td>47</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Colostrum-free pigs given infective brain</td>
<td>28</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>75</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>Controls</td>
<td>22</td>
<td>1*</td>
<td>3</td>
</tr>
</tbody>
</table>

* = contact control.
pigs, and successful transmissions were obtained with a pool of material from outbreaks nos. 1, 2, and 3, and from outbreak no. 10.

The causal agent has been passed serially through two passages in pigs which received colostrum and through three passages in colostrum-free pigs. It may be seen from Table XVI that, of 75 pigs inoculated with infective material successful transmissions were obtained in 24; twelve of the 75 inoculated pigs died from unrelated conditions. Twenty pigs were inoculated with a filtrate and transmission was obtained in four of them. Greater success was found with antibiotic-treated inocula and 20 of 50 pigs showed lesions of encephalitis. The agent could be more easily passed to colostrum-free pigs than to those that had received colostrum; with the former, 16 of 28 pigs were positive whereas with the latter only eight of 47 were successfully infected. The disease was produced in one of four pigs by contact infection. Histologic lesions of encephalitis were not found in control pigs which were treated in a manner similar to pigs given infective material but with normal brain being substituted for the infective material.

Reference to Tables XIV and XV shows that the technique of loading each pig with infective material by inoculating it intracerebrally, intranasally, and orally, was the most successful method of producing infection but it appears to be unnecessary since the results obtained by intracerebral or oral routes alone were nearly as good. Successful transmissions did not occur after intranasal or subcutaneous routes alone, but they were not tested extensively.

Successful transmissions were obtained in piglets about four hours of age and in pigs two and three weeks of age. The best results
were found with newly-born piglets but these were the colostrum-free animals also and this is probably the chief factor contributing to their increased susceptibility.

Generally the histologic lesions found in experimental animals were milder than those of natural cases. Some were very mild and consisted of no more than occasional cuffs and glial nodules, but these lesions were of the same type as those seen in naturally infected animals and differed from them only in intensity. Although there is a possibility that some of these mild changes could have been produced by other causes they were probably produced by the infective agent and they were classified as positive transmissions in the one plus group together with the other pigs with mild but specific lesions. Pigs which showed suspicious changes were classified as negative. Histologic lesions were classified as two plus and three plus when they were of a severity similar to that found in many field cases but changes approximating those of severe natural cases were not observed in experimental animals. Definite lesions were found in the brains of some pigs which did not show clinical disease but these lesions were mild.

Mild changes were found in the brain of a pig that was inoculated with a pool of material which had been stored at -15°C for 14 days and then at -40°C for 280 days and it appears from this observation that the infective agent may be kept well by freezing it.

Rabbits

Brain material, from six field outbreaks and from experimentally infected pigs, was tested for its pathogenicity for rabbits. Thirteen
New Zealand white rabbits were inoculated with Seitz-filtered or antibiotic-treated material; three were inoculated with 1.0 ml of inoculum by the subcutaneous route, six with 0.25 ml by the intracerebral route and four by both routes in each animal. Five of the rabbits were given material that had produced encephalitis in experimental pigs.

Results. The animals were observed for at least three weeks but they remained normal.

Guinea Pigs

Intracerebral and intraperitoneal routes have been tested in four mature guinea pigs. Two were given 0.25 ml of inoculum intracerebrally and two were given 1.0 ml intraperitoneally. They remained normal over a three month observation period.

Mice

Litters of mice, of the Connaught strain, ranging in age from one day to three weeks were inoculated intracerebrally, intraperitoneally, and both intranasally and intracerebrally, with material from nine field outbreaks and from five experimentally infected pigs. The amount of inoculum given intracerebrally was approximately 0.03 ml for the very young mice and 0.1 ml for the older animals; the amount given intraperitoneally was 0.1 ml and intranasal inoculation was done by giving about two drops of inoculum in each nostril. In several instances blind passages with brain material have been made.
Results. No abnormalities were noted which could be attributed to transmission of the swine encephalitis agent.

Chickens

One day old chickens were inoculated by intracerebral or intramuscular routes with 0.1 ml or 0.2 ml of inoculum respectively. They were observed for 10 days but remained normal.

Embryonated Hens' Eggs

Embryonated hens' eggs of appropriate age were inoculated with 0.2 ml of inoculum into the yolk sac, into the allantoic sac, and onto the chorioallantoic membrane; inoculation into the yolk sac was done into two to three day eggs as well as seven day eggs. The eggs were either observed until hatching or material was harvested from them and used for serial blind passages.

Results. No indications of the growth of an infective agent were observed.

Tissue Cultures

Primary cell tissue cultures of the stationary-tube type were prepared from kidneys of pigs which were aged from one week to about six months. The methods used were those described by Madin et al (54). When monolayers had formed they were inoculated with 0.2 ml of a 10 percent brain suspension. The inoculated cultures were observed for periods varying from seven to 20 days. Serial blind passages were made.
Results. No convincing cytopathogenic effects were seen in any of the tissue cultures. Although in some cases changes were noted these were not of sufficient severity or consistency to constitute unequivocal evidence of the propagation of an infective agent.
DISCUSSION

The results of the transmission experiments in pigs show that "Ontario Encephalomyelitis" is caused by a transmissible agent which may be carried serially in pigs, and which is filterable. It may be concluded therefore, on this basis and on the character of the histologic lesions, that the causal agent is a virus.

The failure to infect laboratory animals and eggs eliminates from further consideration as possible causes of "Ontario Encephalomyelitis," Aujeszky's Disease, Rabies, Japanese B Encephalitis, Louping Ill, the equine encephalitides, and Swine Influenza. The strict host specificity found, with pigs being the only susceptible species, is compatible only with the viruses of Hog Cholera and the Teschen Disease group; clearly the virus is not that of Hog Cholera since the pathologic and clinical pictures are quite different. By a process of elimination it must be acknowledged that "Ontario Encephalomyelitis" is either a new disease of swine or that it belongs with the group of diseases consisting of Teschen Disease, Talfan Disease and Poliomyelitis suum of Denmark.

Rather more difficulty has been experienced in transmitting this Ontario disease than has been reported for Teschen Disease (17) (19) (32) but there are reports of difficulty, in passing the latter to experimental pigs (3) (27) (35) (40) (75) (79). These failures to transmit Teschen Disease, which probably depended on the susceptibility of the experimental animals occurred when the latter were older pigs or were drawn from enzootic areas; probably they had
already developed some resistance to the disease. Due to the frequent occurrence of the Ontario disease, it is likely that a similar situation exists in Ontario and this would account for the rather indifferent results obtained in the transmission experiments in which pigs which had received colostrum were used. Further evidence that this was so was provided by the experiments using colostrum-free pigs in which the disease could be produced with considerable success.

Natural, inapparent infections occur in Teschen Disease and neutralizing antibodies have been found in herds in which clinical disease has not been observed (30); also, working with Poliomyelitis suum, Bendixen and Sjolte (4) found that 14.1 percent of animals from infected herds had lesions in their spinal cords when they were slaughtered even though they had not been noticed ill. That inapparent infections occur in the Ontario disease is shown by the presence of histologic lesions in some experimental animals that had remained clinically normal.

Animals experimentally infected with Teschen Disease usually show fairly severe lesions of encephalomyelitis and this is in contrast to the results found here where the lesions in experimental animals were usually mild. The difference may be due, in part at least, to the rapid deaths found in the experiments with the virus of "Ontario Encephalomyelitis."

Based on reports that cortisone either alone or with ACTH will increase the susceptibility of mice and hamsters to the virus of Poliomyelitis (73) (74), a few pigs were given cortisone prior to inoculation but, although very mild lesions were found in two of them,
the results and lesions were not impressive enough to warrant further use of this technique. The experiment, however, was very limited and any conclusion that cortisone does not aid infection would not be valid.

The failure to establish the virus in tissue cultures, has little significance in attempting to assess the identity of the agent. The viruses of Teschen Disease (43) (58) and Talfan Disease (6) have been cultivated in tissue cultures but the diagnostic value of such tissue cultures lies in the detection of neutralizing antibodies in test sera and not in the isolation of the virus in the tissue cultures. In addition, apart from the original isolation of Talfan virus in tissue cultures, considerable difficulty has been experienced in subsequent attempts to cultivate it from field cases (64).

When using a Seitz filtrate for the inoculum the results were rather disappointing in that although characteristic histologic lesions were produced, only four of 20 inoculated pigs were positive. These low figures appear to depend in part on the fact that most attempts were made with pigs which received colostrum rather than with colostrum-free pigs, but probably depend mainly on the failure to get a sufficient amount of virus through the Seitz pad. De Vicente Jordana (7), working with a coliphage and a phage from Bacillus polymyxa, found that unless large volumes are filtered a low proportion of virus is found in the filtrate. For example, he found that all the phage was absorbed on the pad when 10 ml was filtered and that minimal adsorption occurred when 50 ml was filtered. In all the attempts with the Ontario virus only small volumes of suspension were filtered and the amount of virus passing the filter probably was reduced considerably. Harding, Done and Kershaw (28) were
unable to produce experimental infections with Talfan virus after Seitz filtration of the inoculum but they were successful when they used collodion membrane filters.

In summary then, it can be said that "Ontario Encephalomyelitis" is caused by a transmissible agent which will pass bacterial sterilizing filters and is apparently a virus. Of the known viruses causing encephalomyelitis of pigs, its host range is similar only to those of the viruses of Teschen Disease, Talfan Disease, Poliomyelitis suum, and Hog Cholera.
SEROLOGIC INVESTIGATIONS

Sera from pigs recovered from natural infection, and from hyperimmunized animals were tested for neutralizing antibodies against the viruses of Talfan Disease and Eastern Equine Encephalomyelitis. The neutralization tests against Talfan virus were done in tissue cultures of pig kidney by Mr. Huck of Weybridge, England. Doctor Hanson of the University of Wisconsin tested the sera against the virus of Eastern Equine Encephalomyelitis by serum neutralization tests in hens’ eggs.

The sera were collected from the following animals:

Sow with infected litter. This sow was involved in a natural outbreak of "Ontario Encephalomyelitis" and she had shown vague and transient signs of illness. Her litter died and typical lesions of encephalomyelitis were found in them. Two weeks after she was sick, the sow was slaughtered and serum was collected. Histologic lesions of encephalomyelitis were not found in her brain or spinal cord.

Recovered naturally infected pig. This pig was a survivor from a natural outbreak in which all its litter-mates died of "Ontario Encephalomyelitis". It was two weeks of age when it was affected and clinical signs of anorexia and depression were seen without definite nervous signs. Sera were collected before and after an attempt was made to hyperimmunize the pig with 4 ml of a 10 percent suspension of infective brain given intramuscularly. The first sample was taken six weeks after it was sick, and the last sample was collected when it was killed two weeks later. Lesions of encephalomyelitis were not detected in this pig.
Hyperimmunized normal pig. A normal pig was placed in contact with the recovered, naturally-infected pig in an effort to induce a contact infection; the normal pig did not sicken and subsequently it was given a course of hyperimmunizing doses of infective material. It received a total of 9 ml of a 10 percent suspension intramuscularly. Sera were collected before, during and after the hyperimmunizing program. The pig was killed when it was about 16 weeks old and the last serum sample was collected at that time.

Rabbit. A rabbit was inoculated intracerebrally and subcutaneously with infective material and after it failed to sicken it was given a course of hyperimmunizing doses intravenously over a four week period. It received a total of 5½ ml of a supernate of a 10 percent suspension of brain. The serum was collected when it was killed.

Results

The results are given in Table XVII. Both the sow with the infected litter and the recovered natural case had neutralizing antibodies in high titres against the virus of Talfan Disease. The hyperimmunized normal pig showed an increase in its titre of antibodies against the virus of Talfan Disease during hyperimmunization. The result obtained with the rabbit serum was inconclusive. Titres of about 1/20 and above are regarded as specific (33).

Neutralizing antibodies against Eastern Equine Encephalomyelitis virus were not found in any of the sera.
**TABLE XVII**

Results of Serologic Investigations.

<table>
<thead>
<tr>
<th>Serum Sample</th>
<th>Serum Neutralization Titres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Talfan&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sow with infected litter</td>
<td>1/640</td>
</tr>
<tr>
<td>Recovered naturally-infected pig</td>
<td></td>
</tr>
<tr>
<td>1) before hyperimmunizing dose</td>
<td>1/640</td>
</tr>
<tr>
<td>2) after hyperimmunizing dose</td>
<td>1/640</td>
</tr>
<tr>
<td>Hyperimmunized normal pig</td>
<td></td>
</tr>
<tr>
<td>1) before hyperimmunizing doses</td>
<td>1/20</td>
</tr>
<tr>
<td>2) during hyperimmunizing program</td>
<td>1/80</td>
</tr>
<tr>
<td>3) after hyperimmunizing doses</td>
<td>1/80</td>
</tr>
<tr>
<td>Rabbit</td>
<td>inconclusive</td>
</tr>
</tbody>
</table>

N = no neutralizing antibodies.
EEEE = Eastern Equine Encephalitis.
* = Tested by Mr. R. A. Huck, Central Veterinary Laboratory, Weybridge, England.
** = Tested by Dr. R. P. Hanson, University of Wisconsin, Madison, Wisconsin.
Discussion

The finding of a high titre of antibodies against the virus of Talfan Disease in the sow with an infected litter, in a recovered natural case, and the finding of a rise in titre during hyperimmunization of a normal pig, preclude the possibility that this is a non-specific neutralization and clearly indicate that the viruses of "Ontario Encephalomyelitis" and Talfan Disease are antigenically very similar if not the same. Chaproniere, Done and Andrewes (6), on the basis of serum neutralization tests in swine kidney tissue cultures, concluded "that Talfan disease and Teschen disease are caused by identical or closely similar viruses, which are also closely related to or identical with 'Poliomyelitis suum' from Denmark."

From these sets of observations, with the virus of Talfan Disease acting as the link between them, it may be concluded that the viruses of "Ontario Encephalomyelitis", Talfan Disease, Teschen Disease, and Poliomyelitis suum of Denmark, are antigenically closely related and in all probability are identical.
GENERAL DISCUSSION AND CONCLUSION

The combined evidence from the pathology, bacteriology, transmissibility and host range serves to eliminate the known causes of encephalomyelitis in pigs as possible causes of "Ontario Encephalitis" except for those viruses causing Teschen Disease, Talfan Disease and Poliomyelitis suum, which are antigenically closely related or identical. The filtration experiments with the agent of "Ontario Encephalomyelitis" indicate that it is a virus and its antigenic properties confirm the grouping of this disease, based on the pathology and host range, with Teschen Disease, Talfan Disease, and Poliomyelitis suum; there is no tenable evidence against such a grouping.

The classical reports on Teschen Disease describe it as a clinically severe disease which causes an extensive and severe encephalomyelitis, but it is clear that such a definition of the disease is too narrow. In an investigation using four strains of the virus, Fischer (17) noted variations in the pathogenicity of strains of Teschen virus; some produced milder histologic lesions and had a longer incubation period than others. Fortner (19) found that differences in the virulence of different strains occurred as was indicated by variations in the length of the incubation period and the rapidity of death. That the virus of Teschen Disease may lose its pathogenicity for pigs was shown by Mayr (57), who found that after 90 and 91 serial passages in swine kidney tissue cultures the virus was unable to produce clinical disease after intracerebral inoculation.

Perhaps the most striking evidence, proving that Teschen Disease is not the villain that it is claimed to be, is provided by the
studies on Talfan Disease, in which it was found that the disease had appeared surreptitiously in England without the sudden explosive appearance that would have been expected from published data on Teschen Disease. Talfan Disease has a milder clinical syndrome and milder neurologic lesions than those of classical Teschen Disease, but the viruses were shown to be identical or closely related (6) and a comparative study of the neuropathology failed to distinguish between the diseases (11). Good evidence to separate Talfan Disease from Teschen Disease is lacking and it is apparent that Talfan Disease is nothing more than a mild form of Teschen Disease and not a distinct disease entity in its own right. The next few years should see the name Talfan Disease superseded by what appears to be its correct title, Teschen Disease.

Although the evidence necessary to include Poliomyelitis suum of Denmark and "Ontario Encephalomyelitis" under the name of Teschen Disease is not as strong as is desirable, it is considerable, and on the basis of the similarities of the histology, serology and host specificity the grouping of these diseases is logical.

These findings on the relationships of the viruses of Talfan Disease, Teschen Disease, Poliomyelitis suum of Denmark, and "Ontario Encephalomyelitis" strongly suggest "that classical Teschen Disease may be only an extreme manifestation of an infection which is much more widely distributed than has hitherto been suspected." (11). This belief is supported by the diagnosis of Teschen Disease in Madagascar (47)(65) and by the occurrence in Portugal (78) and Holland (80) of porcine poliomyelitides with histologic lesions of the same type as those of Teschen Disease but differing from it in their epidemiology. The
available information indicates that Teschen Disease is no longer confined to Eastern Europe but is widely spread in Europe and occurs in Great Britain and in Northern America.

It is highly probable that the disease observed in Ontario has been present in the United States of America for a number of years. Many comments are present in the literature on unidentified encephalomyelitides of swine in the United States but unfortunately the descriptions are not sufficiently detailed to allow good comparisons with the Ontario disease.

The first report appears to be that of Doyle (12) who found, in 1937, what appeared to be an infectious posterior paralysis mainly affecting sows but it was also noted in suckling pigs. It was mostly limited to pigs of one litter and frequently affected every pig in the litter. The clinical signs varied from a mild ataxia to a complete posterior paralysis and the forelegs were affected in some cases. Foci of inflammation were found in the nerves of the hind limbs and in the white and grey matter of the spinal cord.

In 1943, McNutt (51) briefly described his experiences with an unidentified encephalomyelitis of swine; he was not successful in transmitting it. An editor's note in an American journal (14) states, "Outbreaks of unidentified nervous disorders in swine have been reported by practitioners in widely separated locations in the Corn Belt." An anonymous author (2) pointed out in 1953 that only one suspected case of Teschen disease had been reported in the United States. Eveleth et al (15) also in 1953, found several droves of swine in which symptoms of paralysis were apparent, and although Listeriosis was found in two instances they suspected Teschen Disease in the others but transmission
attempts were unsuccessful. Lastly, McNutt (52) while reviewing the work of Thordal-Christensen on Poliomyelitis suum in Denmark, comments "In the United States, the conditions known as paraplegia would offer a challenge in differential diagnosis."

From these very brief reports, it is apparent that there is present and has been present for a number of years in the United States a disease of swine which is chiefly characterized by paralytic signs and resembles Poliomyelitis suum of Denmark in its sporadic occurrence and mild clinical signs. In all likelihood the disease observed in Ontario and the disease noted in the United States are caused by the same infective agent but this probability awaits a comparison of the agents, particularly of their antigenic nature.

Although further investigations on the serology of "Ontario Encephalomyelitis" are required the results presented in this thesis lead to the conclusion that the disease is Teschen Disease which is presenting itself in a milder form than that classically described for it. Because of the milder nature of the clinical syndrome the disease has previously escaped identification and, if the disease in the United States is the same as that in Ontario, it has become widely distributed throughout the North American continent. It appears that the disease occurs commonly and is of considerable economic importance but support for this impression must await a thorough and active search for the disease in the United States and the provinces of Canada. The greatest aid for such a search would be the establishment of a strain of the virus in tissue cultures to allow diagnosis by serum neutralization tests. This adequate and simple diagnostic test appears to be the most pressing need
at present since before the correct control measures can be enforced a better knowledge of the extent and epidemiology of the disease in North America is required.
A previously unidentified disease of swine occurring in Ontario is described and investigations into its nature are recorded. The disease was first found in November, 1957 and has been observed to occur frequently since that time. The clinical syndrome is characterized by high morbidity and high mortality in young suckling piglets under about two weeks of age with little tendency to cause clinical disease in older pigs although swine up to four months of age have been affected acutely. The signs are those of a disease of the central nervous system and a brief clinical course with convulsions is common in young piglets while older animals and those less acutely affected tend to show ataxia and paresis before convulsions occur.

Specific gross lesions are not found but the histologic lesions characterize the disease as a non-suppurative, non-demyelinating encephalomyelitis and ganglionneuritis. Inclusion bodies are not found. The lesions are typical of those of the viral encephalomyelitides.

Apart from minor differences in distribution and degree, the histologic lesions of "Ontario Encephalomyelitis" simulate closely those described for Teschen Disease, Talfan Disease, and Poliomyelitis suum of Denmark.

The disease has been passed through two passages in piglets which received colostrum and through three passages in colostrum-free piglets. The infective agent is filtrable through an STI Seitz pad and appears to be a virus. Rabbits, mice, guinea pigs, chickens,
embryonated hens' eggs and tissue cultures of swine kidneys are not susceptible to infection.

Sera from a sow with an infected litter, a recovered natural case, and a hyperimmunized normal pig, showed specific neutralization of the virus of Talfan Disease in tissue cultures of swine kidney.

On the basis of the combined evidence from the nature of the histologic lesions, the strict host range, and the antigenic properties of the causal agent, it is concluded that "Ontario Encephalomyelitis" belongs with the group of diseases comprised by Teschen Disease, Talfan Disease, and Poliomyelitis suum. These four diseases do not appear to be distinct disease entities and the available information indicates that they are merely different clinical expressions of a viral polioencephalomyelitis of swine with classical Teschen Disease representing the most severe form and Poliomyelitis suum the mildest form. The disease appears to be widely distributed in North America and, in all probability, it has been present but unrecognized in Canada and the United States of America for a number of years.
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Figure 1. Vascular reaction showing the nature of the cells in the perivascular cuffs, and a mitotic figure showing proliferation in situ. (H and E X1200)

Figure 2. A wide perivascular cuff in the medulla oblongata. (H and E X350)
Figure 3. Perivascular cuffing and glial reaction in the grey matter adjacent to the fourth ventricle of the medulla oblongata.

(H and E X90)

Figure 4. The characteristic cell-type forming the glial nodules; note the central lysis in this nodule. (H and E X900)
Figure 5. Nodular gliosis surrounding a degenerate neuron; a cuffed vessel is present on the right. (H and E X800)

Figure 6. Inflammatory changes in the Gasserian ganglion. (H and E X120)
Figure 7. Inflammatory reaction in the nodose ganglion. These changes are typical of the lesions found in the craniospinal and sympathetic ganglia. (H and E X600)

Figure 8. Perivascular cuffing and glial reaction in the superficial layers of the olfactory bulb. (H and E X200)
Figure 9. Lesions in the parenchyma of the hypothalamus adjacent to the third ventricle. (H and E X200)

Figure 10. Cervical spinal cord of a young piglet, showing lesions in the grey matter about the central canal. (H and E X120)
Figure 11. Lesions in the grey matter of the dorsal horn of the cervical spinal cord of a young piglet. (H and E X200)

Figure 12. Inflammatory reaction in the ventral horn of the lumbar spinal cord of a pig which was about eight weeks of age. (Toluidine blue X90)
Figure 13. Moderately severe meningitis over the cerebellar cortex of a young piglet. (H and E X120)

Figure 14. Meningeal and cortical lesions in the cerebellum of a pig which was about four months of age. (H and E X90)
Figure 15. Meningitis and parenchymal lesions of the cerebral cortex. (H and E X200)

Figure 16. Glial reaction in the region of the hippocampal gyrus of the cerebrum. (H and E X200)
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