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THE ESTABLISHMENT OF ISOLATION UNITS FOR NEWBORN PIGS

and

STUDIES ON AN ENCEPHALOMYELITIS OF NURSING PIGS.

A thesis

Presented to the School of Graduate Studies

of

The University of Toronto

by

THOMAS J.L. ALEXANDER

In partial fulfilment of the requirements

for the degree of

Master of Veterinary Science

1960.
BIOGRAPHICAL SKETCH

Thomas John Lyndon Alexander was born in Cardiff, Wales, on the 7th October, 1930. He was educated at Cardiff High School, Oundle School, and the Royal Veterinary College, London, where, on the completion of his studies in 1954, he qualified for membership in the Royal College of Veterinary Surgeons. After working for one year in a horse practice in Berkshire, he returned to London University to study for the degree of Bachelor of Veterinary Science, which he obtained in 1956. He then worked for one year in a mixed practice in Cornwall before emigrating to Canada to take up the post of graduate assistant in the large animal clinics of the Ontario Veterinary College. This work led him to take up graduate studies in October 1958.
ACKNOWLEDGMENTS

The author wishes to express his sincere thanks to Drs. C.K. Roe, M. Savan and B.J. McSherry for their willing help and guidance; to Profs. R.P. Forshaw and D.L.T. Smith, Dr. A. MacKay and Mr. L. Thackeray for their friendly advice and tuition; and to Dr. K.V. Jubb and Mr. P. Richards, who carried out histopathological examinations of the brains of the experimental pigs used in these studies. The author is also grateful to Dr. H.C. Rowsell and the animal attendants at the Research Farm for their cooperation in the establishment of isolation units; to the Extension Photographic Service for the preparation of the illustrative plates; and to Mr. G.E. Fountain and Mr. W.G. Awrey for their assistance in the examination of blood samples. Finally, he wishes to thank his wife for her technical and typographical assistance, and her unwavering moral support.
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INTRODUCTION

In 1958, Roe and Alexander described a disease of nursing pigs which became known by its salient characteristics as "Vomiting and Wasting Disease". It was first observed in the fall of 1957, reached epidemic proportions in the late spring and summer of 1958, and then waned and disappeared in the winter of that year.

A second condition appeared almost concurrently and caused some confusion as to whether one or two disease entities existed. Although more sudden in onset, in the early stages it was indistinguishable from "Vomiting and Wasting" disease, but whereas the initial signs of "Vomiting and Wasting" disease persisted and became chronic, those of the second epidemic progressed to an acute encephalomyelitic stage which ended in either death or complete recovery.

As the epidemic of "Vomiting and Wasting" disease waned, that of encephalomyelitis flared up, reaching maximum proportions in the winter of 1958 and the spring of 1959. The clinical aspect of this epidemic was described by Alexander, Richards and Roe (1959), and the pathology and the early transmission attempts were described by Richards and Savan (1960).

Attempts to transmit "Vomiting and Wasting" disease in 1957-58 (unpublished work) impressed upon the author the difficulties inherent in experimental transmission of this disease in nursing litters. The value of colostrum-free pigs in such work was made clear by Young and Underdahl (1953) and Haeltermann (1956) and a visit to the veterinary school at Cambridge, England, confirmed the impressions already formed that isolation units for rearing colostrum-free pigs would be an invaluable asset for disease transmission work.
Part (I) of this thesis is concerned solely with the planning, installation and management of isolation units at the Ontario Veterinary College, and Part (II) is concerned with their use in the investigations carried out upon the encephalomyelitis of swine mentioned above.
PART I

The Establishment of Isolation Units for Newborn Colostrum-Free Pigs
The method outlined in this thesis for rearing colostrum-free pigs is a modification of methods developed by other workers. It is simple and straightforward, but an attempt to carry it out without a full understanding of its underlying principles is almost certain to meet with disaster. The following literature review, as well as describing methods employed in rearing colostrum-free pigs, also discusses the underlying principles. Section 1 therefore deals with the immunity of the newborn pig and its relationship to colostrum. Sections 2 and 3 are concerned with rations for newborn pigs and their environmental requirements. Sections 4 and 5 involve applications of the principles outlined in sections 1, 2 and 3 and are concerned with the various methods which have been devised for rearing colostrum-free pigs. Section 6 is an appraisal of the usefulness of isolation units. Finally, there is a resume of the main points brought out in the literature review.

1. **Immunity in the Newborn Pig:**

**HISTORICAL:**

There was considerable speculation in the 19th century upon the nature of immunity in newborn animals, but the only work of importance was that of Ehrlich (1892) who showed that the immunity of a newborn mouse was passively acquired from the dam. In the light of present knowledge it seems remarkable that he failed to realise how his results pointed directly to the milk as being the vehicle for its passage. An exhaustive review of the literature of the 19th century on the subject of immunity in the newborn was written by Famulener (1912).
The first worker to comment on immunity in newborn pigs appears to be Reynolds (1910). He noted that newborn pigs nursing sows which had recovered from hog cholera were immune to hog cholera until they were 5 weeks old. His observations were supported by McArthur (1919) who found that sows which had been vaccinated against hog cholera not only passed their immunity to the first litter born following vaccination, but also to the second and third. He suggested that this immunity was transmitted in the milk throughout the suckling period.

Connaway (1921) stated that he had found high antibody titres both in the colostrum of sows suffering from Brucellosis and in the sera of the litters sucking such sows. He had not found the same high titres in the sera of litters sucking uninjured sows.

A different approach was made by Kuttner and Ratner (1923). They presented the hypothesis that the morphology of endotheliochorial and hemochorial placentas (carnivores, rodents and man) allowed the transference of antibodies from mother to offspring, whereas the morphology of epitheliochorial placentas (pigs and ruminants) did not. This relationship has been discussed more fully by Ratner et al. (1927), Mason and Dalling (1930), Perla and Marmostan (1941), McGirr (1947) and Olsson (1959).

Nelson (1932, 1934) is generally acknowledged to be the first worker to present conclusive evidence that transference of specific antibodies from sows to their newborn pigs occurs only through colostrum. He produced a solid immunity in sows to vaccinia virus, and showed that the suckling young of these sows did not react to challenge with vaccinia virus whereas their colostrum-free litter-
mates reacted with the formation of vesicles. This acquired "suckling immunity" was demonstrable in the young of 6 successive farrowings following a single vaccination.

It has been shown that the immunities in swine to the swine influenza virus (Young and Underdahl, 1949, 1950a,b), to Brucella (Hoerlein, 1952), to Erysipelothrix (Wellman and Heimer, 1957), to Salmonella (Olsson, 1959) and to Escherichia (Speer et al., 1959) are transmitted from sows to their newborn pigs only through the colostrum. This work and the accumulated evidence of many other workers who employed electrophoresis, protein precipitation and serology in their investigations, demonstrates conclusively that the sow's placenta is impermeable to antibodies and that colostrum is the sole vehicle for the transference of acquired immunity in the pig.

The immunity acquired from the colostrum does not persist very long. Connaway (1921) stated that it dwindles "in a few weeks". Nelson (1934) found that it began to decline during the second month and disappeared altogether in the third. Later workers have found that its duration depends on its initial level in the newborn pig's serum. Usually it takes 4-8 weeks to disappear, falling off most rapidly during the first 4 weeks, (Young and Underdahl, 1949, 1950; Hoerlein, 1952; Wellman and Heimer, 1957; Rutqvist, 1958; Olsson, 1959).

The antibody-forming apparatus is incomplete at birth, (Olsson, 1959). Hoerlein (1957) found that he could not stimulate a measurable antibody response in colostrum-free pigs younger than 8 weeks of age. This means that colostrum-free pigs possess no acquired immunity until they are over 8 weeks of age.
MECHANISM OF TRANSFER:

Certain aspects of the transference of immunity from mother to offspring have not been studied in swine, and conclusions can only be drawn from the work in cattle. In cattle it has been shown that the antibodies of the colostrum are derived entirely from those circulating in the dam's blood (Larson, 1958), but in passing from the blood to the colostrum they undergo a change in composition (Smith, 1946). No such change occurs in their passage from the gut to the blood of the calf (Smith, 1948) in spite of this passage being partly intracellular, through the cytoplasm of the epithelial cells of the small intestine. From the epithelial cells the antibodies enter the lacteals, and thence into the thoracic duct, the whole process of absorption taking 1-2 hours, (Comline, Roberts and Titchen, 1951a, b). A similar process probably occurs in swine.

Many workers have noted the absence of antibody from the blood of newborn pigs and its dramatic rise following the ingestion of colostrum. (Connaway, 1921; Moore et al., 1945; Jakobsen and Moustgaard, 1950; Foster et al., 1951; Nordbring and Olsson, 1957, 1958; Wellman and Heimer, 1957; Olsson, 1959). According to Young and Underdahl (1949, 1950), antibodies are demonstrable in the serum of newborn pigs 30 minutes after suckling, reaching maximum levels in 6 hours, the final titres being related to the titres in colostrum. Hoerlein (1952), Rutqvist (1958) and Olsson (1959) reported similar findings.

Normally proteins are broken down into amino acids before being absorbed by the gut. The discovery by Laskowski and Laskowski (1951) that bovine colostrum contains a high concentration of trypsin
inhibitor indicates one means by which colostral antibodies may escape digestion by enteric enzymes. Hill (1956), however, working with laboratory animals, postulated that, in those species receiving antibody through the placenta, gastric digestion began at birth, whereas in those species receiving antibody through the colostrum, gastric digestion was delayed. The work of Barrick et al. (1954) and Nordbring and Olsson (1958) lends weight to Hill's argument. They found that the addition of trypsin inhibitors to orally dosed antibodies had little or no effect on the quantities absorbed. Furthermore, the ability to absorb antibodies from the gut lasts only a short time (Bruner et al., 1949). Nordbring and Olsson (1958) estimated that between 4 and 12% of the total colostral antibody ingested in the first day of life was absorbed by the pig, whereas at 72 hours of age, only 0.2% was absorbed. Speer et al. (1959) found that, even at 24 hours of age, the amounts absorbed were negligible. If proteolytic enzyme inhibitors play any major part in preventing the digestion of colostral antibodies, some other unknown factor must be present to place a time limit on the absorption of such antibodies.

In many species selectivity occurs in the absorption of antibodies by the newborn. In such species, all the ingested antibodies are absorbed by the intestinal epithelium, but some are destroyed before being released into the lacteals, (Rogers-Brambell, 1957). The evidence for selectivity in the pig is conflicting. Barrick, Matrone and Osborne (1954) could observe no marked or consistent rise in the serum gamma globulin levels of one day old pigs after oral dosing with porcine gamma globulin, porcine or bovine serum solids,
or cow's colostrum. Young, Hinz and Underdahl (1955) found that the oral administration of 50 ml. of specific anti-T.G.E. porcine serum to 4 hour old pigs failed to give them any protection to T.G.E. whereas lesser amounts given intraperitoneally gave some protection. On the other hand, Nordbring and Olsson (1958) found that the absorption of porcine serum antibody by newborn pigs following oral dosage was about 4-15% of the total administered. This is only slightly less than the amount of specific antibody absorbed from porcine colostrum. Olsson (1959) demonstrated that the immune globulins in horse serum administered orally to newborn pigs were absorbed and appeared in their sera. The fact that these different reports conflict is probably in itself an indication that selectivity in the absorption of antibodies by the gut occurs in the pig.

THE NATURE OF COLOSTRAL IMMUNITY:

The specific acquired immunity contained in colostrum is not only related to those diseases which have affected the sow clinically. According to Meyers (1936), inapparent infections may result in an immunity as solid as that which follows an overt attack of disease. Such infections are wide-spread. To use Meyer's own words, "the world may be flooded with a sea of inapparent disease agents". In addition, Raffel (1953) believes that the increasing immunity observed in association with age resistance may also be due to a maturation of the defence mechanism of the animal. Whatever process may be involved in the acquisition of immunity, it is certain that the adult sow possesses a strong specific immunity to a wide variety
of infections. She also possesses a variable degree of non-specific immunity. It has not been conclusively proved that this non-specific immunity is transferred, like specific immunity, from dam to offspring through the colostrum, but Kotsche (1957, 1958) and Gwatkin and Annau (1959) suggested, on the basis of their own experiments, that such was the case. The evidence supporting such a postulate is only circumstantial. For example, the properdin system of Louis Pillemer (1955) is in the beta-globulin fraction of serum and possibly, therefore, in colostrum, too. Again, the non-specific bacteriostatic effect of certain anti-proteolytic enzymes in human serum have been demonstrated by Burdon and McRoberts (1953). McCance et al. (1949) have shown that the enzymes present in colostrum pass unchanged into the blood stream of the newborn animal.

Thus the newborn pig obtains from colostrum a resistance, the strength and complexity of which cannot be ascertained accurately. The deprivation of colostrum therefore removes an important unknown and variable factor from disease transmission experiments, and renders the experimental pig more susceptible to infection.

2. Development of Sow-Milk Replacers:

Studies having as their objective the development of a replacer for sow's milk have proceeded along 2 main lines; one, the modification of cow's milk, and two, the preparation of semi-synthetic rations.

Both lines of study have been based, for the most part, on simulating the composition of sow's milk. Table 1 lists values for the composition of sow's milk. Line 1 of this table shows the average
values calculated by Hughes and Hart (1935) from their review of 17 papers published between 1865 and 1933. Line 3 shows the values given by Braude et al. (1947) who were the first to obtain milk samples from sows following the use of oxytocin. Since the sow has voluntary control over milk let down, the samples of Braude et al. (1947) and all the workers coming after them are probably more representative than the samples taken by previous workers.

RATIONS BASED ON MODIFIED COW’S MILK:

The composition of normal cow’s milk is well known. Table 2 lists typical values given by various workers for different breeds of cattle.

A comparison of the composition of sow’s milk and cow’s milk shows that sow’s milk is more concentrated, due mainly to the higher protein and fat levels. The level of protein relative to the other solids is also higher in sow’s milk, approaching 30%, as compared to 25% in cow’s milk.

In spite of these differences, Washburn and Jones (1916) and Newlander and Jones (1935) were able to rear pigs weaned from the sow at 2 days of age, on a ration consisting solely of unmodified cow’s milk. McRoberts and Hogan (1944) showed that, while the findings of these earlier workers were essentially correct, young pigs fed a ration of unmodified cow’s milk were not very thrifty. They concluded that cow’s milk was deficient for the pig during the first few weeks of life.

Several attempts have been made at modifying cow’s milk to render it more suitable for feeding to newborn pigs.
Evvard, Quintin and Glatfelter (1925) compared the effect on 3 week old pigs of feeding unfortified cow's milk, and cow's milk fortified with either eggs, orange juice or tomato juice. When measured in terms of growth rate, cow's milk fortified with eggs produced by far the best results. This is not surprising since the hen's egg, particularly the yolk, has been shown to be an excellent source of the nutrients required by newborn animals. Romanoff and Romanoff (1949) in their review of the composition and nutritive value of eggs, claimed that egg yolk is very digestible, and an excellent source of high quality protein, minerals and vitamins.

Young and Underdahl (1951, 1953, 1955, 1960), who, more than any other workers, have been responsible for the development of successful and practical techniques of rearing colostrum-free pigs, have developed a ration which has been widely quoted. It consists basically of eggs and milk, containing 1 egg yolk per quart of homogenized pasteurized cow's milk. To this is added a mineral salt mixture containing ferrous sulphate, copper sulphate, manganese chloride and potassium iodide. In addition, they inject colostrum-free pigs at birth with vitamin K. With this ration they claim that they can rear a high percentage of colostrum-free pigs with growth rates only slightly inferior to "good doers" on the sow.

Theoretically, their ration appears sound. Homogenization has been shown to increase the digestibility of milk, probably by modifying the casein (Washburn and Jones, 1916) and, although heating decreases the nutritive value of milk, the degree of damage depends upon the temperature used. Pasteurization has only a slight deleterious
effect which is probably outweighed by its bacteriocidal action (Morse, 1913; Daniels and Laughlin, 1920; Evvard et al., 1925; Mapson, 1956). The only obvious alteration which could be made to the Young and Underdahl formula is to increase the butterfat level to make it closer to that of sow's milk. Bovine butterfat, however, does not seem to be tolerated by baby pigs in high quantities and attempts at feeding cow's milk of butterfat levels equal to that of the sow's milk have proved to be unsatisfactory or even disastrous in some cases, (Washburn and Jones, 1916; Evvard et al., 1923, 1925; Sheffy et al., 1951; Young and Underdahl, 1955).

**SEMI-SYNTHETIC SOW-MILK REPLACERS:**

The first successful semi-synthetic milk replacers were formulated for calves. Johnson, P.E. (1940) reported a semi-synthetic milk ration which, while it kept calves alive from birth, produced poor growth rates. Wiese et al. (1947) described a ration which not only supported life but produced growth rates comparable with those of naturally reared calves. Johnson, B.C. et al. (1948) took the synthetic cow's milk formulated by Wiese et al. (1947) and fed it to pigs weaned at the age of 24 hours. The pigs thrived. This appears to be the first report of a successful semi-synthetic sow-milk replacer.

Since 1948 many successful semi-synthetic diets have been devised both for commercial production and for experimental work on the nutrition of baby pigs (Lehrer et al., 1949; Thompson et al., 1952; Catron et al., 1953; Reber et al., 1953; Speer et al., 1954; Crampton et al., 1954; Bauriedel et al., 1954; Cunha, 1957). In most of the reports where semi-synthetic rations have been successfully used to
rear baby pigs, the pigs have first been allowed access to colostrum. Only one report, that by Catron et al. (1953) could be found where such rations had been used to rear colostrum-free pigs from birth.

3. Environmental Requirements of Colostrum-Free Pigs:

Two factors stand out in importance in the environmental requirements of colostrum-free pigs. First is the necessity for relative freedom from environmental pathogens, and second is the necessity for a warm homeothermic environmental temperature.

Section 1 of this literature review, concerning the relationship of colostrum to immunity, makes it apparent that a newborn pig deprived of colostrum is also deprived of its acquired immunity. The necessity for isolation and for strict sanitary precautions in rearing colostrum-free pigs has been emphasized by Young and Underdahl (1951), Catron et al. (1953), Bauriedel et al. (1954), Barrick et al. (1954) and Shuman et al. (1956).

A warm homeothermic environment is necessary because the newborn pig, like the newborn of many other mammalian species, does not possess the ability to maintain a constant body temperature (Brody, 1943). Both Wallach et al. (1948) and Newland et al. (1952) demonstrated a substantial drop in the body temperature of newborn pigs under normal barn conditions. The ability to maintain a constant body temperature gradually develops over the first 2-10 days of life. According to Terrill (1958), even pigs weaned at 1-2 weeks of age need an environmental temperature of 75° to 80° F. The necessity for a warm environment for rearing colostrum-free pigs has been emphasized by Young and Underdahl (1953), Bauriedel et al. (1954),
Done (1955), and Whitehair and Thompson (1956).

4. **Types of Isolation Unit:**

There have been various reports of the successful small-scale rearing of colostrum-free pigs. For example, Bauriedel et al. (1954) reared them in screen-bottomed cages with overhead heat lamps; Whitehair and Thompson (1956) reared them in metabolism cages; and Shuman et al. (1956) reared them in brooder units. However, the only reports in which colostrum-free pigs were reared on a large scale for both experimental and farm repopulation purposes have been by workers who used isolation units based on the same principles as Horsfall-Bauer units, but redesigned specifically to house pigs.

In 1940, Horsfall and Bauer described a box-shaped sealed unit for the individual isolation of infected and control laboratory animals in a single room. Each unit had its own air supply and was big enough to accommodate a small cage. Horsfall and Bauer designed these units for work on highly infectious airborne diseases and, although any manipulation of experimental animals required extreme precautions, they claimed that they were able to maintain the units in close proximity to one another in a battery arrangement without cross-infection. They worked mainly with ferrets but also maintained monkeys and dogs successfully in the units.

Levine and Fabricant (1950) modified the Horsfall and Bauer units specifically for work on infectious airborne disease of chicks. They designed small galvanized iron units whose air supply, in relation to that of the room in which they were housed, was under positive pressure. The accidental passage of airborne infectious agents between
unit and room would be therefore always outwards. They claimed that they were able to maintain chicks with Newcastle disease, chicks with infectious bronchitis and uninfected controls in close proximity without cross-infection.

Young and Underdahl (1953) redesigned the Horsfall and Bauer units specifically for work on infectious diseases of pigs from birth to 4 weeks of age. Their units were originally made of stainless steel and were arranged in batteries. There were no air inlet ducts. Fans placed in the outlet ducts sucked air from the room, through bacterial filters, into the units, and thence out through the outlet ducts. The same box shape was kept, but the inner cage was dispensed with. Instead, a false floor for filtering feces and urine was introduced, along with a complex arrangement of feeding and service doors. A 150 mm. pyrex watch glass provided an observation window.

Since their original report in 1953, Young and Underdahl (1960) have further modified their units. They are now made commercially of fibre-glass in two sizes, the original standard baby pig unit (14 x 14 x 22 in.) and a larger unit (22 x 22 x 23 in.) for raising pigs beyond 4-5 weeks of age, or for maintaining other animals. They state that fibre-glass does not stand up to autoclaving, but it does stand up well to chemical sterilization.

In 1956, Haelterman, employing the basic principles involved in each of the above types of unit, described simpler but equally effective units made out of common galvanized iron garbage cans. They were designed initially for work on transmissible gastro-enteritis in pigs. The pigs lived on expanded metal platforms through which their
feces and urine were able to drop onto flat cones placed at the bottom of the can. A feed pan which could be rapidly replaced when necessary was secured on one side of each can by a metal latch and rubber gasket to keep the unit air-tight. A half-inch copper feeding tube, stoppered on the outside by a rubber bung, penetrated the wall above the feeding trough. The top of the unit was closed by a cellophane sheet suitably secured by strips of inner-tube from an automobile tire. The positive pressure ventilation system designed by Levine and Fabricant (1950) was adopted in preference to the negative pressure system employed by Young and Underdahl (1953). Each unit was supplied with its own air inlet and outlet, the air within the units being under positive pressure.

5. Management of Colostrum-free Pigs:

Colostrum-free pigs can be obtained by catching them at parturition, (Young and Underdahl, 1951, 1953; Bauriedel et al., 1954; Johnson et al., 1955; Done, 1955; and Shuman et al., 1956), by cesarean section (Bauriedel et al., 1954; and Whitehair and Thompson, 1956), or by hysterectomy (Young et al., 1955a, 1957; and Hoerlein et al., 1956).

There appear to be two drawbacks to the first method. The time of parturition is often inconvenient, and, however carefully the pigs are taken, it is difficult to keep them free of contamination. The second and third methods are superior in both these respects. However, cesarean section is a fairly elaborate process involving some danger to the newborn pigs. If suitable apparatus is available, hysterectomies are quicker, simpler, and have more chance of producing viable pigs. Hysterectomy hoods have been specially designed for the third method by Young and Underdahl (1955a), Underdahl and Young (1957) and Hoerlein (1956).

Once in the units, most newborn pigs soon learn to drink
unaided from a trough, (Goodwin, 1958). The findings of Newlander and Jones (1935) and Wells et al. (1940) indicate how much and how often they should be fed. Apparently the undistended stomach capacity of newborn pigs is about 15-50 cc. (average 29.8 cc.), and the frequency with which sows suckle newborn pigs is about once every 1-2 hours, decreasing as the pigs grow older. However, most workers report that feeding three times daily gives satisfactory results, although Evvard et al. (1923) found more frequent feeding produced better growth response. The amount of feed given depends on the concentration of the feed and the capacity of each individual pig. Young and Underdahl (1951, 1953) found that 1 quart of their modified milk formula was enough for 6 pigs for one feeding. Most workers emphasize the need for moderation since overfeeding causes diarrhea and death.

Few authors have mentioned the methods by which they have sterilized their equipment. Young and Underdahl (1953) state that they used chemical disinfection.

6. Appraisal of the Usefulness of Isolation Units:

The following claims have been made for colostrum-free pigs housed in isolation units by Young and Underdahl (1951, 1953, 1955a, 1960), and Hoerlein (1957):

1. A survival rate of 95%.
2. A growth rate superior to that of pigs raised naturally (slower initially, superior subsequently).
3. The provision of ideal experimental conditions for research in (a) nutrition, (b) immunity (e.g.
antibody response), (c) infectious disease.

4. The large scale production of disease-free herds.

At first sight the 5% mortality rate reported by Young and Underdahl compares favourably with the estimates of the Veterinary Investigation Service (1959) of Britain that the average death rate of pigs up to 8 weeks of age in England and Wales from 1956 to 1958 was 25.9%. It also compares favourably with the estimates quoted by Tribe (1954) for Australia (20.9%), Canada (15.9%), New Zealand (25.2%) and the United States (33%). However, this comparison may not be justified since Young and Underdahl (1960) only place in the units newborn pigs which come up to their own arbitrary standards. The findings of Wallach et al. (1948), Newland et al. (1952), Forshaw et al. (1953) and Gwatkin and Annau (1959) indicate that birth weights markedly affect survival rates and growth rates. Hence, if Young and Underdahl were to place every pig born, including the runts, into their units, the survival rates might be substantially lower.

Claims that isolation units are of value for controlled nutrition experiments are justified to the extent that they facilitate the rearing of colostrum-free pigs, result in a dependable survival rate, and preclude many of the factors (e.g. environmental pathogens) which may interfere with the assessment of the results. Furthermore, since colostrum contains various nutrients in high concentration (Perrin 1955), colostrum-free pigs are better suited for nutrition studies than suckled pigs.

Hoerlein's claim (1957) that colostrum-free pigs in isolation units are ideal for the study of antibody response is both justified and qualified by his own work. He showed that colostrum-free pigs
under 8 weeks of age are capable of no measurable antibody response to antigenic stimulation. At this age they are too big for isolation units. Furthermore, colostrum-free pigs are not germ-free (Young et al., 1955a, 1959; Larson and Hill, 1955), so that when they are old enough to be capable of antibody response, the results are not free from interference, for even in the completely germ-free life study of Reyniers et al. (1946, 1949), sterile antigens in the air and food produced antibody responses. However, Hoerlein's claim cannot be discredited entirely. Colostrum-free pigs maintained in a relatively disease-free environment are undoubtedly better for certain immunological research than naturally reared pigs.

Claims that isolation units are of value for controlled experiments in infectious diseases are justified by the work done on T.G.E. by Young et al. (1955b), Haelterman (1956), and Goodwin and Jennings (1959); and by the work done on swine influenza by Urman et al. (1958). However, the claim of Young and Underdahl (1951) that colostrum-free pigs are uniformly susceptible to infection requires modification.

To claim that colostrum-free pigs are uniformly susceptible is to overlook the factor of innate immunity. The comprehensive literature review of Nungester (1954) makes it apparent that the nature of innate resistance is both complex and variable, and its level difficult or impossible to measure. Perla and Marmostan (1941) concluded, also from a review of the literature, that genetic factors contributed considerably to the resistance of the individual to infection, but that little was known about the nature of this resistance.
It seems clear, therefore, that newborn colostrum-free pigs, while being much more susceptible to infection than suckled pigs, probably possess an innate resistance whose strength and complexity is unknown and variable.

The last use for which isolation units are claimed to be of value is for the production of disease-free herds. This claim has been proved largely true by the extensive swine repopulations that have taken place in Nebraska under the direction of Young et al. (1959, 1960). However, this claim, too, must be qualified. The term, disease-free, has been admitted to be a misnomer by Young and Underdahl (1960). Pigs raised in isolation units, and maintained in isolation herds only remain free of infectious diseases which cannot be contracted congenitally and whose sole mode of transmission is directly from pig to pig. Non-infectious diseases and infectious diseases which can remain dormant in the soil, or which are carried by such intermediate hosts as earth worms, may still afflict them. In addition, the growth-promoting effect of antibiotics in pigs in isolation units, demonstrated by Hill and Larson (1955), would appear to indicate that the intestinal bacterial flora of these pigs could not safely be called non-pathogenic. Furthermore, as has been pointed out before, disease-free, colostrum-free pigs can be raised successfully for repopulation work without the use of units. However, such considerations should not detract from the excellent use to which isolation units have been put in Nebraska in the establishment of so-called "disease-free" herds.
7. **Resume:**

The important points brought out by this review of the literature are as follows:

1. The sow's placenta is impermeable to antibodies.
2. Colostrum is the sole vehicle for the passage of acquired humoral immunity from the sow to her newborn pigs.
3. Selectivity is probably exercised in the absorption of antibodies by the intestines.
4. There is a time limit of about 24 hours when antibodies can be absorbed in any appreciable quantities by the intestines.
5. The passive immunity so acquired diminishes rapidly during the first month of life and is negligible by the end of the second.
6. There is a delay in the maturation of the mechanism of active immunity which is more pronounced in colostrum-free pigs than in pigs that have been suckled.
7. Successful but complex semi-synthetic sow milk replacers have been formulated and can be purchased commercially.
8. Cow's milk modified by the addition of egg and mineral salts has been used successfully for rearing colostrum-free pigs in isolation units.
9. Colostrum-free pigs are extremely difficult to raise unless they are kept in isolation from other pigs in a warm, relatively disease-free environment.
10. Basically, two types of successful isolation units have been devised, the fundamental difference between them being their systems of ventilation.
11. Colostrum-free pigs in isolation units are useful for research in nutrition, disease and serology, and for the production of so-called "disease-free" herds.

12. Colostrum-free pigs can be raised with greater certainty, and their experimental value can be enhanced, by the use of isolation units.
THE ESTABLISHMENT, MANAGEMENT AND TESTING OF
ISOLATION UNITS AT THE O.V.C.

Design:

The units described by Young and Underdahl (1953) and Haelterman (1956) were specifically designed for disease transmission work in colostrum-free pigs. With the exception of the ventilation systems their basic principles were the same.

According to Young and Underdahl (1960), fiber-glass units of their design were available from a Nebraska manufacturer, but enquiries revealed that their cost and shipment to Guelph would be prohibitive. The "garbage-can" units designed by Haelterman were reputed to be as effective as the more elaborate units designed by Young and Underdahl, and a local firm agreed to manufacture them at a reasonable cost.

Several modifications of the Haelterman design were made. Figures 1-4 show the final plans and figure 5 shows the component parts.

Since they were to be used for the investigation of diseases which affected pigs up to 6 weeks of age, they were much bigger than the Haelterman units. The limiting dimensional factor was the width of the doors (28 inches) to the rooms where they were to be housed. Their diameter was therefore set at 26 inches and their height at 30 inches.

To prevent direct interference by the imprisoned pig, the lower ventilation tube was placed below the false floor, and protected from contamination with feces by its diagonal inner aperture. A flap
was introduced to prevent the pig from lying in or fouling the feed-trough. The clamps designed by Haelterman for securing the feed-trough in position were replaced, for easier manipulation, by a strip of sprung metal, hinged at one end and secured at the other by a slot. In view of the size of this unit, a central support was designed to extend from the cone to the false floor. On the advice of Haelterman, the feeding tube used in his unit was not included. The units, cones and feed-troughs were constructed of galvanized iron which was both robust and easily disinfected. Because of its rigidity, expanded metal was used for the false floor. The top of the unit was sealed by a transparent plastic sheet held in place by a large elastic band cut from the inner-tube of an automobile tire. The feed-trough gaskets were made of a synthetic material*, which was compressible, non-porous and able to withstand repeated cleaning and sterilizing without losing these properties.

Ventilation System:

The ventilation system was constructed on the following basic principles:

1. The external air inlet had to be at the opposite end of the building to the external air outlet, and so placed that the prevailing winds would be unlikely to carry aerosol infection from the outlet back to the inlet.

2. The air on entering the units had to be -
   
   (a) clean and fresh
   
   (b) free from pathogens
   
   (c) at a thermostatically controlled temperature higher

*Armet Industries Ltd., Guelph, Ont.
than that of the units themselves.

3. There had to be a minimum of 5 air changes per hour, and the air flow had to be readily adjustable.

4. The system had to be air tight and closed.

5. The pressure within the units had to be higher than that of the rooms in which they were housed.

The final plans of the ventilation system have been omitted from this thesis since they were a problem for an engineer. However, figure 6 is a diagramatic representation illustrating the air flow through the system when 5 units are set up in each room. It shows a fan, heating unit and baffle plates placed close to the external inlet. The baffle plates and fan are automatically controlled by a thermostat. The outgoing air ducts (O.D.) are, in reality, directly above the incoming air ducts (I.D.). Both are well insulated and covered with painted canvas to allow disinfection of the entire room.

In spite of this insulation, it was found that the temperature of the air passing through these ducts fell rapidly. There was a temperature differential of up to 20°F. in the air entering the first and seventh units in a room. In order to supply warm air to all the units, thermostatically controlled booster heaters (B.H.), set at 130°F., had to be introduced into the system where the ducts entered each room.

Figure 7 shows the manual stop taps which control the air flow to and from each unit. Half-inch rubber tubes link the air ducts with the units. They are secured at each end by metal brackets. The air circulation within the units is contrary to normal principles.
Air enters at the top and leaves at the bottom. It was hoped that such a system would minimize draughts on the pigs.

Figures 7 and 8 show the plastic covers tightly ballooned by the air pressure within the units. This pressure is higher than that of the rooms so that any chance movement of air carries aerosol infection outward (i.e. from the units into the rooms). The rooms themselves, in relation to the adjoining corridor, are also under positive pressure to minimize entry of aerosol infection from the corridor.

Simple bacteria filters are of questionable value, especially in eliminating viruses. In their germ-free life studies, Reyniers et al. (1946, 1949) found that efficient filters had to be amenable to constant sterilization in situ. Their design was too complicated for the project described here. It was assumed that any pathogens in the fresh air of the research station would be in low concentration, and would be reduced still further in passing over the heating coils of the ventilation system. Therefore, since no attempt was being made to rear germ-free pigs, filters have been omitted from the system.

**Temperature Control:**

In order to raise colostrum-free pigs, the internal temperature of the units had to be between 85-95°F. Contrary to expectations, however, the hot air entering had very little effect on the internal temperature of the units. For test purposes a portable coil-heater was inserted into the ventilation system a few inches from the air inlet of one of the units. It was found that a rise of 100°F. (i.e.
from 80°-180°F. in the temperature of the air entering the unit raised
the internal temperature of the unit by only a few degrees. Due to
the extensive surface of radiation presented by the unit, its internal
temperature was always within 1-2°F. of the temperature of the room.
Although the rooms were themselves thermostatically controlled, the
construction of the building was such that the temperature within the
rooms fluctuated with the outside temperature. It was, therefore,
found necessary to insulate the outside walls of the building with
fiber-glass and the floors of each room with vermiculite, and to fit
each room with heating lamps. A temperature recorder placed in one
of the units showed that these measures were effective. The internal
temperatures of the units could be maintained at 90-95°F., even in the
coldest weather.

Management:

Two guiding principles underlie every phase of the successful
management of colostrum-free pigs:

1. Everything must be done to prevent the lowering of
   their already low resistance.

2. Their environment must be kept as free as possible
   from pathogenic organisms.

The main application of these principles is in the avoidance
of stress and the adoption of sterile techniques. Any factor causing
stress (e.g. overfeeding, irregular feeding, chilling) will lower
the pigs' resistance and result in a high mortality rate. Any break
in the "sterile chain" may result in the introduction of a lethal
dose of pathogenic organisms. For this reason, in the application
of the following methods, every effort was made to approach the ideals of surgical sterility.

METHODS OF OBTAINING PIGS:

The literature review pointed to hysterectomy as being the method of choice. However, because the laws of Ontario forbid the consumption of meat killed within two weeks of parturition, and because no hysterectomy hood was available, cesarean section was used wherever possible.

The drawbacks of obtaining colostrum-free pigs by catching them at birth were found to be more formidable than indicated in the literature review. The difficulty of forecasting the approach of parturition, the inconvenience of night-time farrowings, the prolongation of parturition due to interference by the attendant, and the difficulty of keeping the newborn pigs free from contamination, made this method tedious and impracticable. On the two occasions when it was used, the pigs were washed with disinfectant-detergent* before being placed in the units.

Cesarean section, on the other hand, could be performed at a time more convenient to the attendants, was less time consuming, and resulted in pigs that were surgically sterile. The operation was carried out on the first occasion that milk could be squeezed from the sow's teats. Tranquilizers and an inverted "L" block were used for anaesthesia, and standard surgical techniques were employed. Throughout the whole procedure the sow was handled as quietly as

*"Dettol" (Reckitt & Sons Ltd.); "Phisohex" (Winthrop-Stearn Inc.)
possible. The newborn pigs were wiped with sterile cloths, their
nавels were ligatured, and they were placed in sterile boxes for trans-
port to the units. They were placed into the units through the feed-
trough aperture. From their birth until their death, they were handled
as little as possible, and in every case the attendants wore sterile
rubber gloves.

FEEDING:
The ration used was based on that of Young and Underdahl
(1953, 1955), and was as follows:

1 yolk of egg
5 ml. salt mixture
1.5 quarts (Imperial) of cow's milk (homogenized and
pasteurized).

The composition of the salt mixture was:

49.8 g. FeSO$_4$•7H$_2$O
3.9 g. CuSO$_4$•5H$_2$O
3.6 g. MnCl$_2$•4H$_2$O
0.26 g. KI.

Aquam ad 1 litre.

In the initial experiments, cream was added to the milk
prior to homogenization to make a total butterfat content of 6.5%.
It was found, however, that this caused the pigs to scour more readily.
In later experiments cream was not added.

Although pigs could be successfully raised on this formula
to 4 weeks of age, in later experiments they were weaned onto a pro-
prietary "synthetic" sow milk replacer in the second week of life.
This proprietary feed* not only produced good growth, but was easier to handle, and soured less quickly in the feed troughs. The 3 pigs that were reared to maturity were weaned onto starter pellets** at 3-4 weeks of age, and onto grower ration*** at 6-8 weeks.

The amount of feed given varied with each individual pig. The first feed, given at 6-12 hours of age, was between 2-4 ounces. Thereafter the pigs were fed progressively greater quantities, depending upon the appetite of the individual pig and the state of its feces. The feces had to be watched closely and if any tendency toward softening occurred, the diet was immediately reduced.

The feed-troughs were changed whenever a pig had not eaten all his previous feed, or at least once daily in the first week. Once feeding of proprietary feed had begun, the feed-troughs were changed only when they appeared excessively dirty.

In the preparation of the fortified milk formula, greatest care was taken to prevent contamination. The milk was taken from the churn in sterile jugs, and all feeding utensils (feeding-troughs, flasks, jugs, pipettes) were washed clean in detergent and disinfectant, rinsed and autoclaved between use. The feeding-troughs were wrapped in cloths prior to autoclaving, and were never touched again except by the handle. The feeding process was carried out as quickly as possible. The controls were always fed first, before the experimentally infected pigs.

*Purina Nursing Chow.
**Purina Pig Starter.
***Purina Hog Grower.
CLEANING AND STERILIZING:

After the removal of a pig from a unit, the unit was soaked and scrubbed in disinfectant and detergent, and then rinsed with tap water and allowed to dry. Sterilization was achieved by "steam-jennying" for 15-20 minutes. The top was immediately put on, the unit was installed, and at least 24 hours were allowed to elapse before a newborn pig was placed in it. This allowed time for the unit to dry and for the system to reach equilibrium. It was found necessary to tie the flap over the trough in a vertical position with sterile string to encourage the newborn pig to drink. When the pig reached 4-7 days of age the string could be removed.

Between each experiment the rubber hoses were autoclaved, the plastic covers discarded, and the walls and floors of the empty rooms washed in disinfectant and detergent.

Bacterial Flora:

In spite of the sterile techniques described in the foregoing sections, the pigs reared in the isolation units rapidly developed a gastro-intestinal flora. Six possible sources of this microflora were considered:

1. Contaminated air supply
2. Contaminated feed supply
3. Inadequate sterilization of the units
4. Congenital infection
5. Post natal infection prior to the entry of the pigs into the units
6. Faults in the sterile techniques.
The first five of these were studied.

Three media were used: sheep blood agar, MacConkey agar, and thioglycollate broth. In each case these were incubated for a minimum of 48 hours.

1. Bacterial Examination of the Air Supply:

Approximately 24 hours after installation of certain units, bottles of broth were inserted into their air supply. The air pressure was insufficient to bubble air through the broth, so it was made to blow onto and ripple its surface. The bottles were left in position for 24 hours, removed and incubated.

2. Bacterial Examination of the Feed Supply:

Bacterial examinations were made of:

(a) the milk on its arrival at the College,
(b) the remnants of the modified milk ration left at the bottom of the flask after feeding.

3. Bacterial Examination of the Units:

Two methods were employed.

(a) Instead of being autoclaved, certain used troughs were left in the units and submitted to the same cleaning and sterilization processes as the units. At least 24 hours after reinstallation of the units, approximately 10 cc. of broth were pipetted into these troughs and allowed to remain there for 24 hours. The broth was then removed and incubated.

(b) Sterile swabs were soaked in broth and the strings, trough-covers, mesh floors, and walls (inside surfaces) of installed units were swabbed thoroughly. The broth swabs were then plated onto
blood agar and MacConkey agar and incubated.

4. Bacterial Examination of Pigs at Birth:
   
   Two methods were employed:
   
   (a) Swabs were taken of the rectums, ears and skin of
   many of the pigs immediately after their removal from the uterus.
   
   (b) Four pigs were killed within one hour of birth, by
   ether inhalation. Their flanks were seared with red hot spatulas.
   Sterile instruments were used to excise sections of small and large
   colon which were dropped separately into bottles of broth and incu-
   bated for 96 hours. Small portions of broth were plated out onto blood
   and MacConkey agar after 48 and 96 hours of incubation.

5. Bacterial Examination of the Possibility of Post Natal Infection
   Prior to Entry to the Units:
   
   (a) Blood and MacConkey plates were left open and trans-
   ported with the piglets from the operating theatre to the units. The
   plates were then closed and incubated for 48 hours.
   
   (b) The skin and ears of one litter of pigs were swabbed
   immediately prior to being placed in the unit.
THE EFFECTIVENESS OF THE UNITS
(OBSERVATIONS AND DISCUSSION)

Design:

The units achieved the purposes for which they were designed. They were large enough for raising colostrum-free pigs in individual isolation, up to 5 weeks of age. Beyond that age, both the air supply and the feeding-trough capacity became inadequate, and the accumulated excrement and spilled food rose dangerously close to the air outlet. Furthermore, pigs over 5 weeks of age tended to jump up and push off the plastic covers.

Isolation proved adequate for the disease under investigation. Controls housed in the same room as infected pigs remained uninfected. The plastic covers provided an excellent window for viewing both the pigs and their feces and vomitus, which were retained satisfactorily by the cones.

The units had certain drawbacks. The expanded metal platforms were hard on the feet of newborn pigs. The feet of a few pigs became so sore that they were reluctant to move, and although the feet of most of them rapidly hardened, those in Exp. 9 in which the platforms were covered with muslin, were noticeably more lively than those in other experiments.

The expanded metal platforms were also hard to lie on and the pigs tended to climb onto the flaps covering the troughs for greater comfort. In so doing, many of the strings holding the flaps upright were broken. Since most pigs under 3-4 days of age were unable to solve the problem of raising the fallen flaps, the broken strings
usually had to be repaired, thus adding to the possibility of contamina-
tion.

The air supply was adequate, but it will be seen later that in experiment 6 the pressure was barely sufficient to ventilate two units set up in series. Rubber hoses of greater diameter (e.g. 2 inches) might remedy this. The fans in the ventilation system were not linked up to the emergency electricity supply at the research farm, so that during electrical failures of long duration the covers had to be removed. It was found that newborn pigs could survive power failures of several hours, but older pigs used up the available oxygen in a very short time.

**Survival Rates:**

The pigs placed in the units have been used almost exclusively for disease transmission experiments. Only 3 of them have been allowed to survive beyond 6 weeks of age. Thus, it is impossible to make an accurate estimate of the survival rates. However, table 3 shows the number of pigs placed in the units and the percentage which proved to be of experimental value.

One litter has been omitted from the table. It was obtained by cesarean section, which was carried out as a demonstration for a short course at a time which, of necessity, had to be fixed long in advance. The piglets were premature, very weak and unable to stand. They all died within 2 days of birth.

Six pigs which, through lack of space, were placed in the units in pairs have also been omitted from the table. These pigs continually fought, nudged each other, and sucked each other's umbilicus.
Their feet became excessively sore, and the pigs which became dominant overfed, scoured and died. The dominated pigs were killed. One died of broken ribs and a ruptured liver, another died of hemorrhage through the naval, and another was so badly bruised it was unable to stand.

Column 1 of table 3 lists the 12 experiments carried out in the units plus a preliminary trial. Fourteen litters were involved. Of these, 2 were born naturally and 12 were obtained by cesarean section. Two sows died, one of metritis and one of post-operative shock. The other 12 sows made uneventful recoveries.

Columns 3, 4 and 5 are each subdivided into 2 sets of figures headed A and B. Set A represents the number of pigs treated in the manner stated at the head of the column, and set B represents those which were of experimental value, that is, those which either died as a direct result of the inoculation, or which survived and were healthy when killed at the end of the experiment. Thus, the difference between rows A and B represents the number of pigs which died of other causes (e.g. bacterial septicemia). The percentages at the foot of each column represent the efficiency of the units in providing colostrum-free pigs for experimental purposes.

The percentages in columns 3 and 4 are remarkably close, and that of column 5 is somewhat lower. The cause of this lower percentage probably lies in the fact that this column includes the weakest pigs of each litter (at least 2 were very small runts). It was found almost impossible to rear runts. The biggest pigs at birth appeared to survive better than their smaller litter mates. Thus, if the 2 runts are omitted from the final estimation, the percentage of column 5
rises to 84%. However, even as it stands, with the runts included, the efficiency of the units in providing colostrum-free piglets in individual isolation for disease transmission experiments has been about 86%. Many of the pigs included in this estimate were still very young when killed by the experimental disease, and some of them would almost certainly have died of other causes if they had been left uninoculated. On the other hand, it must be remembered that all the pigs in columns 3 and 4 were subjected to a rigorous inoculation technique which often involved anaesthesia and intra-cranial inoculation. These stresses probably lowered their resistance. Thus, it would not appear over optimistic to expect a similar or even higher survival rate if the pigs were simply being reared for repopulation work, especially if the precedent of Young and Underdahl (1960) were followed and all the smaller pigs were excluded at birth. Almost certainly, the mortality rates would be lower than those quoted previously in the literature review for pigs born and reared naturally.

**Feeding:**

The mineral salt mixture gave a bitter taste to the modified milk ration, and it was found that newborn pigs were tempted to drink at an earlier age when it was omitted. It was therefore usually omitted from the first feed. The milk ration also tended to cause scouring and had to be fed very carefully.

The commercial semi-synthetic ration rarely caused scouring. In addition, it was more easily stored and prepared than the modified milk formula; it did not sour as quickly; the pigs seemed to like it since they ate it ravenously; and it seemed to give a boost to the pigs when
they were weaned onto it. This may have been due to the antibiotics it contained. Hill and Larson (1955) found that colostrum-free pigs reared in isolation units showed better average daily gains when receiving daily supplements of chlortetracycline, than when no antibiotic was supplemented.

No routine records were kept of growth rates, so it is impossible to compare them with naturally reared pigs. The pigs which were reared to 4 or 6 weeks of age, however, appeared thrifty and strong. Figure 9 shows one such pig (approximately 3 weeks of age) feeding at the trough.

The hemoglobin values recorded in table 4 are some indication of the effectiveness of the diets in supplying iron, copper and vitamins. The pigs listed as normal are control pigs and pigs inoculated with infected material which failed to show clinical signs of disease. The pigs listed as infected are those which showed clinical signs. All blood samples were taken immediately prior to euthanasia.

The relevant point of interest arising out of this table is that the values for the healthy pigs rise significantly as the pigs grow older, reflecting the adequacy of the diet. This table (including the differences in the values of healthy and affected pigs) will be discussed more fully in Part II.

**Source of Bacterial Flora:**

Table 5 shows the results of the investigations into the source of the intestinal flora of the pigs reared in the isolation
Bacteria of the coli-aerogenes group were recovered only once, from the remnants of the milk ration left in the bottom of a feeding flask after feeding. Streptococci were recovered from several different sources, and in 11 cases they were alpha-hemolytic. All the other bacteria recovered were probably plate or air contaminants.

The investigations have shown that the pigs possess no aerobic bacteria at birth and derive their enteric streptococci from several sources. However, the investigations have failed to reveal the source of bacteria of the coli-aerogenes group. Larson and Hill (1955), who made a close study of the microflora of pigs in isolation units, concluded that the primary source of this microflora was pasteurized cow's milk. Their conclusions are supported by the bacteriological findings in part II of this thesis. Coliform bacteria were recovered from all the pigs which had drunk milk, but not from pig 248 which never learned to drink and died of starvation. The eggs contained in the modified milk ration may also be a source of coliform bacteria. Reyniers et al. (1949) found that hens' eggs often contained Escherichia coli and were a common source of contamination in their germ-free life studies.
At the onset of these investigations, the epidemic of "vomiting and wasting" disease, mentioned in the introduction, was over, and cases were rarely encountered. The epidemic of encephalomyelitis also appeared to be waning, but outbreaks were still fairly common. The clinical signs of "vomiting and wasting" disease, and the clinical signs and pathological lesions of encephalomyelitis had been described by Roe and Alexander (1958); Alexander, Richards and Roe (1959); and Richards and Savan (1960). The latter had attained limited success in transmitting the encephalomyelitis from field cases to nursing pigs, and they had suggested, mainly on the basis of the histopathological lesions, that it was a form of Teschen disease. Its close serological relationship with Talfan disease was later reported by Richards (1960), who also reviewed the literature on the pathology and serology of Teschen disease, Talfan disease, and epizootic paresis. In the following pages an attempt is made to review those aspects of the three diseases which have not been reviewed by Richards and which are relevant to the investigations described in this thesis.
LITERATURE REVIEW OF TESCHEN DISEASE

Historical:

The name, Teschen disease, is derived from a region along the borders of Czechoslovakia and Germany called Tesin where an epidemic broke out in swine in 1928-29. According to Kaplan and Meranze (1948), Klobouk had already noted a similar disease in Moravia in 1913, and according to Testi (1950), a similar disease had been observed in Italy in 1907. Most authors, however, acknowledge Treffny (1930) as being the first worker to describe the disease, and Klobouk (1933) as being the first to transmit it and to demonstrate its probable viral nature.

It has been described under many different names (e.g. porcine virus encephalomyelitis, infectious pig paralysis, Bohemia pest, meningoencephalomyelitis suum, encephalomyelitis enzootica and ansteckende Schweinelähmung), but the name, Teschen disease, appears to be the most widely used.

In the ten years following 1929, the disease appeared to spread throughout Czechoslovakia, but unfortunately the changing fortunes of that country have rendered the papers published at that time relatively inaccessible. International barriers to the dissemination of disease broke down during World War II and the disease spread from Czechoslovakia into Germany and France (Fortner, 1942). Since the war it has been reported in Italy (Venturi, 1948), Spain (Palacios, 1952), Portugal (Tropa, 1954), and Madagascar (Filet and Verge, 1952).

In 1955, Bendixen and Sjolte described a poliomyelitis of Danish swine, which Thordal-Christensen (1959) later called epizootic
paresis, and in 1957, Harding, Done and Kershaw described a transmissible polioencephalomyelitis of swine in Britain which they called Talfan disease. Later, Teschen disease, epizootic paresis and Talfan disease were shown to be related serologically (Chaproniere et al., 1958).

The presence of Teschen disease has never been confirmed on the North American continent, and was thought not to occur here. (Kaplan and Meranze, 1948; Horstmann, 1952; Jones, 1958). However, as long ago as 1937, Doyle, in the U.S.A., described an infectious paralytic condition of pigs in which the histopathological lesions closely resembled those of Teschen disease.

Clinical Signs in Field Cases:

The clinical signs of "classical" Teschen disease, epizootic paresis in Denmark, and Talfan disease in Britain, differ slightly and are therefore described under separate headings.

CLASSICAL TESCHEN DISEASE:

Many authors have described the clinical signs of Teschen disease, but the reports of Kaplan and Meranze (1948), and of Jones (1958), are the most comprehensive descriptions in English and, except where otherwise stated, the following outline is based upon these.

The incubation period is not known but is thought to vary between 3-31 days. All ages from 5 days to adult are affected, but in enzootic districts, young pigs around weaning age, and newly introduced pigs suffer most. According to Slanina et al. (1956), clinical signs in pigs under 2 weeks of age are rare. However, in enzootic areas entire herds composed of pigs of all age groups occasionally become
When the disease first appears in an area, about 50% of the pigs exposed to the infection become clinically ill (Klobouk, 1931). Later, it may appear in successive waves several months apart, eventually affecting most or all of the animals in a herd. In enzootic areas, it may be sporadic, affecting only individual pigs.

The mortality rate is about 50-60% but occasionally may be 100%. Those pigs that die directly of paralysis do so in the first week. In pigs that die later, death is usually due to secondary pneumonia.

The clinical signs have been described as occurring in three stages, a prodromal stage, a stage of nervous excitement and a paralytic stage. The first two stages, however, often are barely noticeable or absent altogether (Testi, 1950).

The prodromal stage, which is most commonly seen in sows (Slanina et al., 1956), may last from a few hours to 6 days. It is characterized by fever, lassitude, anorexia and slight ataxia. Vomition is rare and has only been mentioned by Kaplan and Meranze (1948).

The fever and anorexia may persist into the second stage, which lasts 2-6 days. The second stage is characterized by nervous excitement which varies both in severity and expression, and may include one or more of the following signs: hyperaesthesia, generalized muscular tremors, excessive chewing, circling, tonic-clonic spasms, and generalized convulsions brought on spontaneously or by handling or noise. When these convulsions occur, the pig is often standing. It froths at the mouth, smacks its lips and grinds its teeth, shows
nystagmus of the eyes, falls over, paddles with its legs for a few minutes, then lies quietly, and finally the signs subside and the pig appears normal again. Alternatively, it may remain prostrate and in a coma for many hours.

The third stage, usually gradual in onset, is the paralytic stage. Paralysis is the dominant and most constant clinical feature of Teschen disease. It is usually ascending, affecting first the hind and then the forelimbs, and it affects mainly the motor neurons, although loss of cutaneous sensitivity may sometimes occur. The clinical signs vary, depending upon the degree and distribution of the paralysis. Thus, some pigs may adopt a dog-sitting position, walking on their front limbs only. Others may suffer complete flaccid paralysis of the neck, tongue, and mandible, or merely an alteration of voice.

During this paralytic stage, the temperature is normal or subnormal and, although constipation is common, the appetite is usually good. Provided the paralysis does not prevent respiration or the ingestion of food, complete recovery may follow in from 2 weeks to several months. In some cases, however, a residual paresis and muscular atrophy may persist permanently.

EPIZOOTIC PARESIS IN DENMARK:

The following description of the clinical signs of epizootic paresis is based on the reports of Bendixen and Sjolte (1955) and Thordal-Christensen (1959).

The disease in Denmark is essentially a spinal motor paresis of growing weaned pigs. Mature pigs and pigs under 4 weeks of age are rarely affected. Outbreaks are never explosive, as described in Teschen
disease. On the contrary, on affected farms, individual pigs are affected sporadically over a period of 3-4 months. The mortality rate is about 6% of clinically affected pigs.

The prodromal stage and stage of nervous excitement are mild and rarely present. The only consistent sign is a spinal motor paresis, varying from slight ataxia with recovery in a few days, to severe motor paralysis lasting several months.

The maximum degree of paresis is reached by the second day of illness. Except in the most severe cases, some movement of the limbs remains and pigs lie in sternal recumbency, with complete sensitivity of the whole body surface. Appetite and defecation remain normal, although, occasionally, paralysis of the bladder occurs.

TALFAN DISEASE IN BRITAIN:

The following description of the clinical signs of Talfan disease is based on the report of Harding, Done and Kershaw (1957), who described 6 herd outbreaks. Their clinical description is poor, but it is the only one that could be found in the literature.

The disease occurs mainly in suckling pigs over 2 weeks of age and in pigs immediately following weaning. However, one outbreak has been described in pigs of 6-8 months of age. Clinical signs occur sporadically in small groups of pigs within a herd, over a period of weeks or months. Both the morbidity and mortality rates are low.

Typically, the picture is one of pronounced ataxia and flaccid paralysis which is sometimes sensory as well as motor. Some affected pigs walk backwards, some hold their heads lopsidedly, and some become recumbent. "Fits", which progress to coma and death, or to incoordi-
nation, have been mentioned but not described.

No significant reduction in appetite or fever occurs and recovery is common. However, it is not always complete and, in some cases, convalescent stunting occurs.

**Clinical Signs in Experimental Cases:**

The clinical signs in experimental cases are basically the same as those in field cases, and so, in the following paragraphs, only points of interest or difference are noted.

**TESCHEN DISEASE:**

Many workers have described the clinical signs in experimental cases of Teschen disease. The best descriptions are by Fortner (1942), Gard (1947) and Horstmann (1952).

The incubation period varies widely, from the minimum of 4 days recorded by Horstmann (1952), to the maximum of 34 days recorded by Fortner (1942). Horstmann found that both the incubation period and the duration of illness were longer following intranasal inoculation than following intracranial inoculation, and were longer still following oral inoculation.

Most workers used recently weaned pigs and growing pigs as experimental animals. Verge et al. (1951) found that pigs of 8-10 kilograms were the most susceptible.

The experimental disease takes the same clinical course as the naturally occurring disease; however, Fortner (1942) observed the stage of nervous excitement only in about 50% of the pigs he infected. Horstmann (1952) reported a 30% recovery rate.
EPIZOOTIC PARESIS IN DENMARK:

The ages of experimental pigs used for transmission purposes in Denmark range from 1 to 6 months. The incubation period varies between 9 and 17 days. The clinical signs produced are very similar to those reported in field cases. Thordal-Christensen (1959) observed a prodromal stage similar to that of Teschen disease, and he reported that convulsions, although rare, did sometimes occur. He attributed the dyspnea which sometimes occurs in conjunction with paralysis of the bladder, to urine retention.

TALFAN DISEASE IN ENGLAND:

The report of Harding, Done and Kershaw (1957) appears to point to a resistance which increases with age. Typical signs and lesions were produced in 10 out of 26 cases inoculated under one month of age, whereas lesions but no clinical signs were produced in only 3 out of 20 pigs inoculated when over one month of age. The oldest pig in which they were able to produce lesions was 7 weeks of age.

The incubation period of Talfan disease varies between 12 and 16 days. Clinical signs rapidly intensify to a maximum within 24 hours of onset, and in some cases they persist for 10-14 days. The clinical signs are similar to those observed in field cases.

Necropsy Findings:

No significant gross post-mortem lesions have been noted in any of these 3 related conditions. However, the histopathological lesions in the central nervous system (CNS) have been described by many authors. They have been reviewed and discussed by Richards and Savan (1960) and Richards (1960).
Experimental Transmission and Pathogenesis:

Brain tissue from clinically affected pigs was used by Klo-bouk (1933) in the first successful transmission of Teschen disease, and such tissue has been used for routine transmissions by all later workers.

Many routes of transmission have been tried but most workers have favoured intracranial inoculation. Fortner (1942), Gasparini and Nani (1955) and Kersting and Pette (1957) transmitting Teschen disease, and Harding et al. (1957) transmitting Talfan disease, found the sub-dural (lumbar) route satisfactory. Kersting and Pette (1957) reproduced Teschen disease by inoculating into the sciatic nerve.

Teschen disease has also been reproduced by using intra-muscular, intravenous and intraperitoneal inoculation (Fischer, 1958). The success, however, has been variable. Using colostrum-free piglets, Kutsche (1958) claimed 100% success with both intraperitoneal and intramuscular inoculation, whereas, using naturally reared pigs, Horstmann (1952) could only produce subclinical lesions in a small percentage of cases.

Two routes of inoculation which have been used consistently and which may reflect the natural transmission of the disease, are the oral and intra-nasal routes. Fortner (1942) favoured the intra-nasal route above all others, but Horstmann (1952), comparing the different routes, obtained 100% success by intracranial inoculation, 73% success by oral dosage, and 66% success by intranasal inoculation.

Fortner (1942) succeeded in 3 out of 29 attempts in transmitting the disease by placing susceptible pigs in direct contact with
infected pigs. He also reproduced the disease using feces collected from affected pigs in the prodromal and paretic stages of the disease. Gard (1947) and Hecke (1958) showed that, following oral dosing of infected brain material, some pigs (but not all) excreted virus in their feces for at least a week, beginning on the day following dosing. Patocka et al. (1955) could find no virus in the feces following intracerebral inoculation.

Hecke (1958) found that the virus was absorbed throughout the entire digestive system and accumulated in high concentration in the regional lymph nodes on the second day following oral dosing. He confirmed the finding of Horstmann (1952) that an asymptomatic viremia occurred on the third to fifth day after oral dosage, at which time the virus could be recovered from tissues in various parts of the body.

Hecke (1958) also found virus in the olfactory bulbs in a very small percentage of pigs following intranasal inoculation.

Patocka et al. (1952) and Buck and Serres (1957) have shown that, at the beginning of clinical signs, there is very little virus in the CNS but that a maximum concentration is reached at the onset of paralysis. The concentration decreases rapidly until on the fifth to seventh day of paralysis it is absent altogether. Fortner (1942) found that he could produce clinical infection using brain tissue diluted 1:1,000.

The accumulative evidence of all these workers indicates that the pathogenesis of naturally occurring Teschen disease resembles that postulated for poliomyelitis of man by Sabin (1956). The virus
is carried in the feces of a percentage of infected animals. It is absorbed throughout the whole digestive tract and gains access to the regional lymph nodes. Then a viremia occurs which leads to the involvement of peripheral nerve ganglia and an ascending spread through the central nervous system. This hypothesis is supported by the chronological histopathological studies of Kötsche (1958) and Fischer (1958).

Hecke (1958) thought that, in a few cases, the virus might also reach the central nervous system via the mouth, pharynx and cranial nerves, or via the nose and olfactory nerves. Fischer (1958), however, pointed out that, although lesions were always present in the olfactory bulbs 24-48 hours after intranasal insufflation, spreading through the CNS in 6 days, they were never present in the olfactory bulbs in naturally occurring cases, or following intramuscular, intravenous or oral inoculation.

Hecke (1956, 1958) and Mayr (1957) have shown that silent subclinical infections are common, perhaps more common in some areas than clinical infections. These silent infections stimulate the production of neutralizing antibodies. Hecke suggested that in these cases the virus reached the lymph nodes but failed to gain access to the nervous system. He warned that such animals were a great epidemiological danger, explaining the invisible spread of the disease from herd to herd without apparent infection.

**Hematology:**

Pedini et al. (1955) described a slight fall in hemoglobin and red blood cell count in infected animals. They noted a marked
decrease in white blood cells after subdural inoculation. This leucopenia changed to a neutrophil leucocytosis with the onset of paralysis.

**Resistance of the Virus:**

Gralheer and Fischer (1958) showed that the virus of Teschen disease remained stable at 0°C at pH 4-11 for 72 hours. It was inactivated by a temperature of 60°C in 20 minutes, but it could be preserved in CNS tissue in a dry-ice chest (Patocka *et al.*, 1952; Jones, 1958).

Fortner (1942) showed that it resisted post-mortem putrefaction for 6 days, pickling at 6-10°C for 21 days, and drying at room temperature for 23 days. It survived in 50% glycerin at 0°C for 20 months. Gard (1947) showed that it resisted ether.

Fortner (1942) found that formalin, choramine and kerosol-sulphuric acid effectively destroyed it. Patocka *et al.* (1952) found that alkalinity increased its virulence. Jones (1958) stated that it was active between pH 2.5 and pH 13, but its virulence was increased between pH 8 and pH 11.
MATERIALS AND METHODS

Thirteen transmission experiments were carried out using the colostrum-free pigs and isolation units described in Part I. Most of the details of the first four experiments have been described by Richards (1960). However, the clinical signs and hematology were not discussed and are included with the details of the last 9 experiments in this thesis.

The first transmission experiment failed (Richards 1960). Transmission material for the second experiment was obtained from a field outbreak in which pigs in 4 of 6 litters, about 10 days of age, suffered a severe and acute attack of the disease. The morbidity and mortality rates within the affected litters were about 90 per cent. The affected pigs showed depression, anorexia and vomition for a period of 24-36 hours before nervous signs became apparent. The brains of necropsied pigs showed microscopic lesions typical of viral encephalomyelitis. One of these brains was used in the second transmission experiment.

The second experiment proved successful and the infective agent was serially passaged in the third and fourth experiments. It had thus undergone 3 serial passages at the onset of Experiment 5.

Materials:

The transmission materials used were brain tissue, feces and urine, from normal and affected pigs.

The brain and feces were prepared as 10% and 20% suspensions, respectively, by grinding with physiological saline in TenBroeck tissue grinders. Antibiotics were added to the suspensions and to the urine,
and inoculation was carried out as soon as possible after preparation.

The choice of antibiotic used was determined by the results of sensitivity tests carried out on bacteria recovered from materials saved for transmission purposes. In nearly every case, chloramphenicol was indicated as the drug of choice and it was added, except where otherwise stated, so that each ml of suspension contained 20 mg chloramphenicol. When no bacteria were recovered from a tissue, a combination of penicillin and streptomycin was added so that each ml of suspension contained 200 I.U. penicillin and 20 mg streptomycin.

The dose of brain suspension given to each pig was 5.0 ml (i.e. 0.5 g brain tissue). Except in Experiment 10, 0.5 ml was injected intracranially, a few drops were inoculated intranasally, and the rest was given by oral dosage. This combination of routes will be referred to as "all route" inoculation. In Experiment 10, the relative amounts given by each route were varied.

The dose of feces suspension varied slightly, depending on the quantity available. In most cases it was 1.0 g per pig in 5.0 ml physiological saline, and it was either given by the combined oral and intranasal routes or by "all routes" inoculation in the same relative quantities described above.

The dose of urine for each pig was approximately 6.0-8.0 ml. Of this 0.5 ml was given by intracranial inoculation, a few drops were inoculated intranasally, and the remainder was given orally.

Techniques

Inoculations (except in Exps. 1 and 13) were carried out on the first or second day of life. For intracranial inoculation, ether
anesthesia was used, and the inoculum was injected well into the brain tissue through a trephined hole in the left temple area.

Blood was collected in tubes containing an anticoagulant immediately prior to euthanasia from healthy and affected pigs. Total white blood cell counts, differential white blood cell counts, hemoglobin and blood urea nitrogen estimations were carried out.

A gross post-mortem examination was carried out on every pig. Brain and sometimes cervical cord were removed for sectioning. Whenever nervous tissue was to be saved as transmission material, the brain was incised longitudinally, the left half was removed aseptically and stored at approximately -35°C, and the right half was fixed in formalin for sectioning.

The bacteriological technique was simple, and limited to culturing for aerobic bacteria on sheep blood agar and MacConkey agar plates. The plates were examined after 24 and 48 hours incubation. In most cases, naked eye examination sufficed to broadly classify the colonies but whenever a doubt arose, bacterial smears were made and examined microscopically. The brain, heart blood, liver, kidney, small intestine and coiled large intestine were cultured routinely in every pig.

Criteria for the Diagnosis of Successful Transmission:

Typical histopathological brain lesions were regarded by Richards (1960) as being pathognomonic, and indicative of successful transmission. However, although the presence of such brain lesions was the best single criterion for a positive diagnosis, their absence (in the experiments described here) did not necessarily indicate a
negative transmission. A combination of other factors sometimes provided the basis for a diagnosis of encephalomyelitis even when no brain lesions were found.

It will be shown later that the clinical signs and, to a lesser extent, the gross post-mortem appearance of the experimental pigs which were affected with viral encephalomyelitis, were distinct enough to be differentiated from those of pigs suffering from other accidentally introduced infections.

If a group of pigs from the same litter were inoculated at the same time, in the same way, with the same dose of material; and if they all became ill on the same day, showing characteristic clinical signs; and if, at necropsy, specific brain lesions could only be found in half of them, it seemed reasonable to make a positive diagnosis for the whole group. However, if any doubt arose, for example, if the clinical signs or gross post-mortem lesions were atypical in one of the pigs of the group, a negative diagnosis would be made for that pig.

Transmission Experiments:

OBJECTS:

The 9 experiments described here were a direct continuation of the first 4 (Richards, 1960), which showed that the disease could be transmitted by oral or by intracranial inoculation. The first object of these experiments was to confirm the findings described by Richards and to continue the serial passage of the infective agent.

The second object was to determine whether transmission occurred when an infected pig was placed into direct contact with a
healthy pig, and if so, whether such transmission occurred via the
feces, the urine or by aerosol droplets.

PLAN:

Although the methods used could be planned in advance, the
sequence, with the exception of the first experiment, depended upon
the number of pigs in each litter. Thus, the experiments, which are
presented here in the order in which they were carried out, may appear
to be somewhat haphazard. For this reason, at the end of the presenta-
tion an attempt is made to categorize the various sections of each
experiment into groups related to the objects. In the interests of
both brevity and clarity the experiments are presented in outline
form.

EXPERIMENT 5

Objects:

1. To determine whether pigs up to 4-5 days old were susceptible
to infection. (It was necessary to know this before transmis-
sion could be attempted by direct contact or aerosol droplet
infection.)

2. To carry out a fourth serial passage of the infective agent.

Materials:

A. Uninfected brain tissue.

B. Infected brain tissue from Exp. 4.

Methods:

Eleven pigs (201-211) were divided into 5 pairs and a single
control. "All route" inoculation was used. A control pig (201) was
inoculated with material A at 5 hours of age, and the pairs were
inoculated with material B at the following ages: 5 hours (pigs 206, 207), at 42 hours (pigs 204, 205), at 66 hours (pigs 202, 203), at 90 hours (pigs 210, 211), and at 118 hours (pigs 208, 209).

EXPERIMENT 6

Objects:
1. To determine whether transmission occurred from an infected to an uninfected pig by direct contact or by aerosol droplet infection.
2. To carry out a 5th and 6th serial passage of the infective agent.

Materials:
Pooled brain tissue from affected pigs 210 and 211 (Exp. 5).

Methods:

Figure 10 shows the way in which the units were arranged. Six units (units 1 and 2 in room 7; units 2 and 3, 4 and 5 in room 6) were linked up in 3 pairs, the second unit of each pair receiving its air supply from the first via a 3 inch-long tube. Contrary to normal practice the air entered the second units just below the false floor and left at the top.

Eleven pigs (221-231) were used. At the start, 1 pig was placed in each unit. The 3 pigs (223, 224, 226) which were in the first unit of each pair, and thus in air contact with the 3 pigs (222, 225, 227), were inoculated with infected brain suspension by "all routes". At the onset of clinical signs, 3 pigs (229, 230, 231), were moved into direct contact with the sick pigs. Two pigs
(228, 221) were left as uninoculated controls. Their units were placed close against the units containing affected pigs.

EXPERIMENT 7

Objects:

1. To determine whether feces taken at necropsy from affected pigs contained infective quantities of the causative agent.
2. To carry out a 7th and 8th serial passage and in so doing to test the susceptibility of the litter.
3. To confirm the direct contact transmission which had occurred in experiment 6, and to attempt another transmission by direct contact.

Materials:

A. Brain tissue from pig 231 (Exp. 6).
B. Feces from pig 231 (Exp. 6).

(Pig 231 contracted the disease by direct contact).

Methods:

Six pigs were used.

Two pigs (241, 242) were inoculated with brain suspension. One of these (241) was inoculated by the standard "all routes" method and the other (242) was dosed orally only.

Two pigs (243, 244) were inoculated with feces suspension. One of these (244) was inoculated by the standard "all routes" method, and the other (243) was dosed orally only.

Two pigs (245, 246) were left uninoculated. One of these (245) remained as an uninoculated control, and the other (246) was placed in direct contact with the infected pig (241) 48 hours after
inoculation, and then moved in with the infected pig (242) when pig 241 died. It was thus in direct contact with clinically sick pigs for 4½ days.

EXPERIMENT 8

Objects:
1. To determine whether urine taken at necropsy from the distended bladders of affected pigs contained infective quantities of the causative agent.
2. To test the susceptibility of the litter used and to compare the susceptibility of colostrum-free pigs with pigs which had received colostrum.

Materials:
A. Pooled brain tissue from pigs 223 and 226 (Exp. 6).
B. Pooled urine from pigs 223 and 226 (Exp. 6).

Methods:
Nine pigs (247-255) were used. Seven of these pigs (247-253) were placed in the units at birth and 2 (pigs 254, 255) were left to suck the sow overnight. However, she would not suckle them. She was therefore milked manually and 10 ml of her colostrum were given orally to each pig prior to being placed in the units next morning.

Two pigs (248, 249) were dosed with urine orally and intranasally, two (250, 251) were dosed with urine by "all route" inoculation, and four (252, 253, 254, 255) were dosed with the brain suspension by "all route" inoculation. One pig (247) was left as an uninoculated control.
EXPERIMENT 9

Objects:

To collect fresh fecal samples from infected and control pigs at frequent recorded intervals following oral inoculation.

Materials:

Pooled brain tissue from pigs 210 and 211 (Exp. 5). (This had proven infective in Exp. 6).

Methods:

A double layer of sterile muslin was secured firmly over the platforms of the units in order to filter urine and retain feces. Eight pigs (261-268) were put in the units. Six of them (pigs 261-266) were dosed orally with brain suspension. Two (pigs 267, 268) were left as undosed controls.

Approximately 15-30 minutes after each feeding, the tops were removed from the units and any feces retained by the muslin were bottled, labelled and stored at -35°C. This procedure was repeated until all the affected pigs had died.

EXPERIMENT 10

Objects:

1. To test by "all route" inoculation, pooled fecal samples for the presence of the causative agent.

2. To raise pigs for later "age resistance" experiments. (These also acted as uninoculated controls in this experiment).

3. To test the susceptibility of the litter.
Materials:

A. Pooled brain tissue from affected pigs 210 and 211 (Exp. 5).
   (This tissue proved infective in Exps. 6 and 9.)
B. Pooled fecal samples from Exp. 9.

   The bacteria cultured from each fecal sample (collected in Exp. 9) were tested for antibiotic sensitivity. Eight samples were selected on the results of these tests, on the freshness of their appearance, and on the time of their collection in relation to the onset of clinical signs. They were pooled in 4 groups:

   1) from the 2 control pigs (267, 268).
   2) from 2 affected pigs (261, 266) collected 24 hours prior to the onset of clinical signs.
   3) from 2 affected pigs (263, 264) collected at the time of onset of clinical signs.
   4) from 2 affected pigs (262, 264) collected 6 hours after the onset of clinical signs.

Methods:

Twelve pigs (271-282) were divided into pairs. One pair (281, 282) were left uninoculated, and 5 pairs were inoculated by "all routes" in the following manner: Pigs 271 and 272 were given B1; pigs 273 and 274 were given B2; pigs 275 and 276 were given B3; pigs 277 and 278 were given B4; and pigs 279 and 280 were given A. (The dosage of feces varied, depending on the amount available, from 0.7 g to 1.3 g).
EXPERIMENTS 11 AND 12

The objects in these experiments were the same and therefore they are described together.

Objects:

1. To test (by oral and intranasal inoculation) for the presence of the causative agent in fecal samples collected in Exp. 9.
2. To test the susceptibilities of the litters.
3. To rear pigs for later "age resistance" experiments. (These pigs also acted as uninoculated controls in this experiment).

Materials:

A. Pooled brain tissue from pigs 210 and 211. (This tissue had already proven infective in Exps. 6, 9, 10).

B. Pooled fecal samples from Exp. 9. Twelve samples, chosen on the same merits as those reported in Exp. 10, were divided into 5 groups:
   1) from 2 control pigs (267, 268).
   2) from affected pig (266), collected at 19 hours and 12 hours prior to the onset of clinical signs.
   3) from affected pig (262), collected at 17 hours prior to and at the time of onset of clinical signs.
   4) from 3 affected pigs (263, 265, 266), collected at 6-11 hours after the onset of clinical signs.
   5) from 3 affected pigs (261, 262, 263), collected at 14-21 hours after the onset of clinical signs.

Methods:

A total of 20 pigs (301-305, 307-319, 321, 322) were used
in the 2 experiments. Four pigs (305, 307, 316, 317, i.e., 2 pigs from each litter) were inoculated with material A by "all routes".

Six pigs (308, 309, 311, 319, 321, 322) were left uninoculated.

Ten pigs (301, 302, 303, 304, 310, 312, 313, 314, 315, 318) were divided into pairs and inoculated orally and intranasally with fecal suspensions in the following manner:

1) Two pigs (310, 318) were given material B1.
2) " " (303, 304) " " " B2.
3) " " (301, 302) " " " B3.
4) " " (312, 313) " " " B4.
5) " " (314, 315) " " " B5.

EXPERIMENT 13

Object:
To determine whether pigs of 2 weeks, 3 weeks and 4 weeks of age were as susceptible as their litter mates had been at 1-2 days of age.

Materials:
Pooled brain tissue from pigs 210 and 211. (This material had already proven to be infective in Exps. 6, 9, 10, 11 and 12).

Methods:
Unfortunately, due to shortage of accommodation, 6 of the pigs retained from previous experiments had to be paired up in 3 units. They fought violently and 5 of them died. Thus, instead of the 8 pigs as planned, only 3 (281, 308, 309) were available for this experiment.
Pig 309 was inoculated at 2 weeks of age.
" 308 " " 3 " " " 
" 281 " " 4 " " " .

Although "all route" inoculation was used, the relative dosages were altered. One ml (instead of 0.5 ml) was inoculated intracranially into the 4 week old pig, and 0.75 ml was inoculated intracranially into the 3 week old pig.

Summary (Categorized):

Tables 6 and 7 group the various sections of each experiment into categories related to the objects.

A total of 76 pigs were used in Exps. 5-13. Fifteen of these were left as controls (i.e. uninoculated or inoculated with material taken from normal pigs). Several of the pigs served dual purposes (e.g. pigs inoculated with infected brain tissue served both as experimental animals for serial passage and as infected controls testing the susceptibility of the litters; and pigs inoculated at older ages served as uninfected controls in their earlier life).

In an attempt to confirm the findings of Richards (1960), infected brain was administered by "all route" inoculation into 18 pigs of 0-2 days of age, and 9 pigs at older ages. Seven pigs were inoculated by the oral route alone from 0-2 days of age. The infective agent was carried from its third serial passage (Exp. 4) through an additional 5 serial passages.

In an attempt to find how the infective agent passed from pig to pig, 4 healthy pigs were placed in direct contact with affected pigs and 3 were placed in aerosol contact, 4 were dosed with urine and
16 were dosed with feces from affected pigs.

In order to establish whether pigs over 2 days of age were as susceptible as their litter mates had been in the first 2 days of life, 9 pigs were inoculated by "all routes" as follows: 2 at 66 hours, 2 at 90 hours, 2 at 118 hours, 1 at 2 weeks, 1 at 3 weeks and 1 at 4 weeks of age.

Richards (1960) described the first 4 experiments carried out in the units, but he omitted the hematology and clinical observations from his description. In these first 4 experiments, brain material from affected pigs was inoculated into 13 pigs by "all routes", into 6 pigs by the oral route, and into 9 pigs by intracranial inoculation alone. There were 16 control pigs. The results that follow will include the hematology and clinical observation from the first 4 experiments along with those of the last 9.
RESULTS

Transmission Attempts:

Tables 6 and 7 list the results of all the transmissions attempted.

Table 6 shows that, of 27 pigs inoculated by "all routes" with brain tissue obtained from affected pigs, 26 developed characteristic clinical signs, but only 16 (59%) showed typical lesions in the CNS. (The one pig which failed to show clinical signs had been very weak from birth and died soon after inoculation). Similarly, of 7 pigs inoculated with brain tissue by the combined oral and intranasal routes, all 7 developed characteristic clinical signs, but only 4 (57%) showed typical histopathological brain lesions.

It has been reported already that 8 serial transmissions were accomplished. Each transmission produced characteristic clinical signs and lesions.

Table 7 lists the results when transmissions were attempted by direct contact, by air contact, and by the inoculation of urine or feces from affected pigs. It shows that successful transmission was accomplished only by direct contact and by oral and intranasal inoculation of feces.

Of the 4 healthy pigs placed in direct contact with affected pigs, 2 developed the disease, showing typical clinical signs and lesions.

Four of the 9 pigs inoculated orally and intranasally with feces developed characteristic signs, and the brains of 2 of them contained characteristic lesions. Both the inocula (B3, B5) given
to these pigs contained feces from pig 262, whereas the pooled inocula used on other pigs did not.

The attempts at transmission by "all routes" inoculation of feces met with disaster. All but one of the pigs became progressively more depressed following the inoculation and died within 2-4 days. In all cases, in spite of the inclusion of suitable antibiotics in the inocula, coliform bacteria could be cultured from the brain, and necrosis and yellow-brown staining could be observed around the site of inoculation.

**Clinical Signs of Viral Encephalomyelitis:**

At least 3 times daily, each pig in the units was carefully observed through the plastic cover. It was only in the terminal stages when live pigs were removed for euthanasia and necropsy that closer examinations were carried out.

The following description is based on 50 cases which were diagnosed with reasonable certainty. It excludes observations on other pigs which may have been suffering from the disease but which showed neither distinct clinical signs nor characteristic histopathological lesions.

The records have been divided into 2 groups. Group A comprises 37 pigs which contracted the disease by "all route" inoculation, and Group B comprises 13 pigs which contracted the disease by combined oral and intranasal inoculation, or by direct contact. Table 2 shows that both the incubation period and the duration of illness were longer in Group B than in Group A. The figures for the duration of illness are not representative because many of the pigs were not
allowed to die of the disease, but were killed in its advanced stages.

**PRODROMAL STAGE:**

A distinct prodromal stage preceded the onset of nervous signs in all but 1 of the 50 cases. It was rapid, almost sudden in onset, and consisted of 4 salient signs: depression, which was usually pronounced; anorexia, which was sometimes only partial to begin with, but always became complete by the second day of illness; constipation, which occurred in all but 4 of the cases; and vomiting, which occurred in 26 cases, and varied from profuse vomiting to persistent retching of green mucus.

In this prodromal stage, a characteristic clinical picture was presented. At one feed a pig would appear healthy and ravenously hungry. At the next it would be depressed, disinterested, and hollow flanked, and standing with its head bowed, its feet together and its tail hanging limply. Occasionally, a fit of retching would convulse its whole body, and pools of green or yellow vomitus would be seen on the cone. In the hours that followed, it would become rapidly dehydrated, oblivious to outside stimuli, and so weak that it would rock forward to take weight on its nose.

**NERVOUS STAGE:**

The onset of nervous signs was gradual and often difficult to detect. In most of the pigs in Group B, they began with a constant twitching of the ears, or repeated clonic spasms of the neck muscles which gave a bird-like pecking action to the head. This also, but less commonly, occurred in Group A. More commonly, in Group A the first nervous signs were generalized muscle tremors, or ataxia, or
paresis or a combination of these. A pronounced dyspnea was common in the early stages, sometimes persisting until death. Both inhalation and exhalation were forced and jerky, either slow and deep or rapid and shallow. In five pigs, the nasal passages became blocked with mucus, causing respiratory stertor. The flanks of many pigs, at first hollow, later became bloated and excessively tense. Cyanosis of the snout and extremities became obvious at some time during this stage.

Paresis of the limbs manifested itself by a tendency in many pigs to squat awkwardly in sternal recumbency and an inability to rise beyond the knees. Others stood with their limbs unnaturally placed. In 5 cases, pigs were observed to walk rapidly backwards, finally sitting like a dog. This paresis appeared to be mainly motor, for even in terminal stages when the limbs were paralyzed, most pigs responded to pin pricking at any point on the body.

Sternal recumbency progressed to lateral recumbency. Although hypoaesthesia was difficult to detect, at least 5 pigs reacted to sudden noise by paddling and squealing furiously for several minutes. The squealing was abnormal in both pitch and intensity.

Champing of the jaw was rare. Nystagmus occurred in 4 laterally recumbent pigs. The only nervous signs shown by some pigs were progressive paresis and paralysis, which were difficult to differentiate from muscular weakness. Temperatures were only taken prior to necropsy. Most were then normal or sub-normal. Only 2 were slightly above normal (103.5°F).
OLDER PIGS:

The incubation periods, course of the disease and clinical signs of the pigs inoculated at various ages up to 118 hours reflected no developing age resistance.

The pigs inoculated at 2 weeks, 3 weeks and 4 weeks of age, developed typical prodromal signs, following incubation periods of 3, 4 and 3.5 days, respectively. The pig inoculated at 2 weeks developed characteristic nervous signs and died. The pig inoculated at 3 weeks recovered its energy and desire to eat after 24 hours, but developed a mild transient posterior ataxia and severe pharyngeal paralysis which lasted for 3 days. It became frantic in its efforts to swallow, attacking the trough and mouthing and splashing the feed furiously. On the fourth day it was able to drink slowly and on the fifth day it had recovered completely. The pig inoculated at 4 weeks suffered a mild illness. It appeared unable to drink for 2 days and developed a barely perceptible posterior ataxia. Recovery was complete on the fourth day.

RECOVERIES:

In addition to the above pigs, 2 others recovered after illnesses lasting 4-5 days. One of these was pig 246 (Exp. 7) which contracted the disease by direct contact. The other was pig 254 (Exp. 8) which had been dosed with colostrum. Both pigs developed typical prodromal signs. Pig 254 developed mild nervous signs, but pig 246 showed no nervous signs at all.

All 4 pigs were killed for necropsy when recovery was fully established, and characteristic lesions were found in their brains.
Clinical Signs of Other Conditions:

Table 3 shows that 86% of the pigs in individual isolation were of experimental value. The remaining 14% died of causes other than viral encephalomyelitis. One pig (248) died of starvation, one pig (247) died of anoxia due to a fault in the ventilation system, and 2 pigs (221, 222) suffered from suppurative polyarthritis and became so lame that they were killed. Staphylococci and streptococci were cultured from the affected joints. The rest appeared to have died from bacterial septicemia and/or bacterial meningitis.

Septicemia occurred following invasion of the blood stream by coliform bacteria. Diarrhea was usually a premonitory sign. A pig so affected would lose its appetite, become increasingly depressed, lie down and die within 6-24 hours of the onset of clinical signs. Cyanosis of the extremities was common but vomiting rarely occurred.

Bacterial meningitis either occurred as a result of septicemia or as a result of the intracranial inoculation of contaminated materials. In the latter, the incubation period was very short, never more than 24 hours. In both cases the course of the disease was rapid, death occurring within 6-24 hours of the onset of clinical signs. No separate prodromal or nervous stages could be noted. The mucous membranes of the eye became very injected, and exophthalmus was common. Nervous signs varied. In some cases they were barely perceptible, and in others they were pronounced. Violent convulsions, circling, hypermotility, nystagmus and opisthotonos were observed.

Necropsy Findings:

The gross post-mortem picture of pigs which remained healthy
until they were killed was always the same. The stomach was full of white or yellow curd and sometimes a little gas; the small intestine was full of pale yellow non-gassy liquid; the large intestine was full of pale yellow, fairly firm feces; and the bladder was either empty, or it contained up to 10 cc of urine. The kidneys appeared normal.

The gross post-mortem picture of pigs which were diagnosed as having died of the viral encephalomyelitis (a total of 35 in the last 9 Exps.) was also fairly constant. Externally, the carcass appeared dehydrated, and there was cyanosis of the nose and feet, yellow-green froth around the mouth, an accumulation of wax at the corners of the eyes, and no wetting of the perineum. The stomach was usually bloated with gas (29 pigs). It rarely contained much ingesta but in at least 22 pigs it contained a quantity of green mucus. In some pigs this green mucus could also be found in the oesophagus. The ingesta in the small intestines was liquid (29 pigs), (deep green (15 pigs) or intense yellow (12 pigs), and very gassy (20 pigs). In only 5 pigs were the feces soft. In all the other pigs they were very firm and dark. The kidneys of 20 pigs contained white-orange calculi, and the bladders of 20 pigs were greatly distended with urine (over 15 cc).

Characteristic histopathological lesions were only found in 35 (70%) of the 50 pigs upon which the clinical description was based. It was noted that in those pigs in which the disease took a slow course, (e.g. where recovery occurred), brain lesions were always present, and were usually pronounced. However, in those pigs which suffered a peracute attack of the disease (e.g. following intracranial
inoculation of 1 day old pigs), brain lesions were often minimal or absent.

The gross post-mortem picture of experimental pigs which died of other causes varied, but in only 1 or 2 cases could it be confused with that of pigs affected by viral encephalomyelitis. Usually the large intestines were full of dark or pale yellow liquid. Occasionally calculi were found in the kidneys.

Hematology:

Table 9 shows the total white blood cell counts of affected pigs which were showing characteristic nervous signs (usually terminal) when the blood samples were taken, and of normal healthy pigs under 2 weeks of age. There is a statistically significant difference between the total white blood cell counts of healthy pigs and pigs infected by oral and intranasal inoculation. However, there is no significant difference between the counts of healthy pigs and pigs infected by "all route" inoculation, and no differences can be noted in the differential white blood cell counts (table 11).

Table 4 shows the hemoglobin values of healthy pigs and of pigs affected with viral encephalomyelitis. Comparing the hemoglobin values of normal and infected pigs during the first week of life, the difference of 2.12 vs. 0.81 (6.85 vs. 8.97 g Hb) is found to be statistically significant (t observed = 2.63, exceeded the required t (DF = 7) of 2.36). Comparing the change in hemoglobin levels with age, the increase in values up to 3 weeks is found to be highly significant in the normal group but no significant change is observed in the infected group.

Table 10 shows the values obtained for blood urea nitrogen estimations. The values in affected pigs, especially in the affected
pigs of group B, are in some cases considerably higher than the values in healthy pigs.

Bacteriology:

Sixty-two pigs were raised in the units in the last 9 experiments. Of these, 3 controls were reared to adult, leaving 59 which were examined bacteriologically at necropsy. Coliform aerogenes bacteria were recovered from the gastro-intestinal tract of all but one (248). This pig never learned to eat and died of starvation. The bacteriological findings in this pig were as follows: Non-hemolytic Staphylococci, alpha- and beta-hemolytic Streptococci and Bacillus species were grown from the intestines and hemolytic Staphylococci were grown from the heart blood.

The coliform bacteria varied from pig to pig, and included hemolytic, non-hemolytic, mucoid and non-mucoid types. Alpha-hemolytic Streptococci were recovered from the intestines of 32 pigs, and Pseudomonas were grown from the intestines of 1; Proteus swarmed over 3 plates.

Of the 14 pigs which were clinically healthy when killed, no aerobic bacteria were cultured from any organs other than the intestines, but of the 45 pigs which died or were sick when killed, coliform bacteria could be cultured from all of the organs of 20 and from some of the organs of 7.

In Experiment 10, where 8 pigs were injected intracranially with feces, coliform bacteria were recovered from the brains of 5, (in 1 of which there was also alpha-hemolytic Streptococci) and Staphylococci were cultured from another. There was no growth from one.

No bacteria were cultured from the brains of 16 pigs which showed clinical signs of viral encephalomyelitis and in which characteristic histopathological lesions were found.
DISCUSSIONS AND CONCLUSIONS

Nature of the Causal Agent:

The causal agent has been transmitted through 8 serial passages. It has retained its pathogenicity after 3 months storage at -35°C and it has resisted chloramphenicol, penicillin and streptomycin. Although Richards and Savan (1960) were unable to propagate it in tissue culture, they were able to filter it through bacterial filters and to demonstrate that the histopathological brain lesions which the filtrate caused were non-suppurative and typical of viral encephalomylitides. It seems reasonable to conclude that the infective agent is a virus.

The clinical signs and histopathological lesions indicate that the virus is neurogenic, and the retention of sensory responses until the terminal stages point to its predisposition for motor neurons.

Richards (1960) and Richards and Savan (1960) have shown that serologically and histopathologically the disease in Ontario is closely related to Teschen disease and Talfan disease, and unrelated to Aujesky's disease, which it resembles clinically (Gordon and Luke, 1955; Shope, 1958).

Clinically it differs from the classical descriptions of Teschen disease in (1) age incidence, (2) period of incubation, (3) duration of illness, (4) frequency of vomition, bloat and cyanosis, (5) absence of residual paralysis or any impairment of nervous function in recovered pigs, and (6) relationship of clinical signs to medullary localization as opposed to spinal and cerebellar localization in Teschen disease, (Manueledis et al., 1954).
The serology, however, overrides the clinical differences, and it would seem safe to assume that the virus causing the disease in Ontario is identical to, a variant of, or closely related to that causing Teschen disease, Talfan disease and epizootic paresis of Denmark.

Pathogenesis:

The foregoing experiments showed that, as in the case of Teschen disease, the virus can pass from a sick pig to a healthy pig by direct contact, and that at least one mode of this transmission is the oral and intranasal ingestion of infected feces.

In pigs infected by combined oral and intranasal inoculation the first specific nervous signs were in the neck and the ears, and the most consistent signs were bowel stasis and dyspnea. These signs can be correlated with the high concentration of lesions observed in the medulla, and indicate that, if the virus reaches the brain via the peripheral nerves, as suggested in the literature review of Teschen disease, it does so mainly by the autonomic nervous system, and mainly, for the reasons stated previously, by the motor nerves of that system. If this is true, then the paralytic dysphagia which occurred definitely in pig 308 (Exp. 13) and probably in others as well, may indicate that in this pig the virus reached the brain via the cranial nerves, after absorption through the pharynx.

Paralysis of the autonomic nervous system may explain the bowel and bladder stasis, which in turn would account for the intense yellow-green colour of the ingesta, its gassy nature, and the retention of urine. The distention of the bowel and bladder with gas and
urine results in the bloated appearance seen in many affected pigs.

The absence of histopathological lesions from the brains of pigs which suffered acute fulminating attacks of the disease led to the hypothesis that when the central nervous system was rapidly overwhelmed with a high concentration of virus, insufficient time elapsed for visible lesions to develop.

The reports of several workers support a similar hypothesis for classical Teschen disease. Fortner (1942) stated that he found typical brain lesions in chronic cases of the disease, but that he was unable to find them in acute cases. Larski and Szaflarski (1956) could only find lesions in 90% of the 270 pigs which they inoculated although all the pigs showed characteristic clinical signs. Hecke (1958) showed that high concentrations of the virus sometimes occurred in regions of the central nervous system where no lesions could be observed.

Clinically, affected pigs appeared to become rapidly dehydrated, probably as a result of the vomiting and anorexia. The hemoglobin values and total white blood cell counts (tables 4, 9), which were both higher in affected pigs than in healthy pigs, may reflect a hemoconcentration resulting from the dehydration. If so, table 9 indicates that this hemoconcentration was only pronounced in pigs which contracted the disease by oral and intranasal inoculation. This was probably related to the relatively long duration of illness in this group, (table 8).

The hemoconcentration, in association with dyspneic hypoxia, may have been partly responsible for the peripheral circulatory failure
and the cyanosis of the extremities. It may also have been the cause of the marked uremia (i.e., pre-renal uremia) observed in some of the pigs in the units and in many field cases of the disease, (Alexander et al., 1959). By causing anuria and urine concentration it may also have been responsible for the calculi observed in the kidneys of many affected pigs. These calculi were probably urates. Madsen et al. (1944) described similar calculi in an uremic condition of newborn pigs, and Yusken et al. (1959) described urates in the kidneys of newborn pigs affected with transmissible gastro-enteritis. In the experiments described here, calculi were also occasionally observed in pigs which died of septicemia and which scoured severely prior to death. The cause of calculi in these conditions may be the same in every case, namely, dehydration, hemoconcentration and urine concentration.

Clinical Signs and Immunity:

The clinical signs in experimental cases closely resemble those reported for field cases, (Alexander et al., 1959). The absence of colostrum in experimental cases undoubtedly allowed earlier invasion of the tissues by coliform bacteria from the gastro-intestinal tract than would occur in field cases. However, the effect of these bacteria on the clinical signs must have been small since no differences could be noted between these pigs and the 16 from the brains of which no bacteria could be recovered.

The mild form of the disease observed in the two pigs inoculated at 3 weeks and 4 weeks of age parallels that observed in pigs of similar ages in the field, and indicates a rapidly developing age
resistance. This resistance is probably not of an acquired humoral nature, but is more likely associated with innate changes in the tissues of growing pigs. That humoral antibodies play a major role in the immunity of the young pig to this disease is apparent from the importance of colostrum. The techniques in the transmission experiments carried out by Richards and Savan (1960) on nursing pigs, and those carried out in colostrum-free pigs in the isolation units were similar, but the results were quite different. Richards and Savan (1960) were only able to produce subclinical infection in a small percentage of the nursing pigs which they inoculated, whereas the great majority of colostrum-free pigs used in the units showed pronounced clinical signs and died of the disease. The pigs used by Richards and Savan (1960) were older than most of the pigs used in these experiments, so the difference is perhaps not as marked as it would first appear. However, the pigs inoculated in Experiment 13 were the same age as those used by Richards and Savan, and here a more direct comparison is possible.

Additional evidence in support of the protection provided by colostrum in this disease is provided by Experiment 8. Here 2 pigs (254, 255) were dosed with colostrum at 24 hours of age prior to inoculation. They both showed clinical signs but one recovered. This was the only pig to recover of all those inoculated by "all routes" in the first week of life. Furthermore, since at 24 hours of age the pigs' ability to absorb colostrum has greatly decreased, these pigs would probably have gained considerably more protection had they been dosed with colostrum soon after birth.
Similar observations on the relationship of colostrum to Teschen disease have been recorded by Kötsche (1957, 1958).

The presence of specific antibodies in a high number of sows in Europe, due to the widespread nature of inapparent infections, probably accounts for the low incidence of Teschen disease in nursing pigs, especially in pigs under 3 weeks of age, and the high incidence in weaners and growing pigs. In this country there appears to be a rapidly developing age resistance, and the only outbreaks that occur are in nursing litters. It is assumed that the sows suckling these litters possess no circulating antibodies against the disease.

The history of this disease and so-called "vomiting and wasting" disease stimulates some speculation. The coincidence of 2 such epidemics overlapping in such a way makes it difficult to believe that they are unrelated. Their morbidity, mortality rates and age incidence are the same. The first clinical signs in both diseases are anorexia, vomiting, depression and constipation. In the encephalomyelitis these signs are more sudden in onset, and after 1 to 4 days, distinct signs of nervous derangement supervene. "Vomiting and wasting" disease takes a more chronic form, no nervous signs or lesions can be observed, and affected pigs waste away, becoming permanently emaciated. The simplest hypothesis is that both epidemics were caused by the same virus which increased in virulence and neurogenic tendencies from the first appearance of "vomiting and wasting" disease in 1957, to the peak period in the epidemic of encephalomyelitis in 1959. If such were true, it would make the clinical differences between this and Teschen disease even more marked. However,
until it is proved otherwise, it is best to regard the two diseases in Ontario as being separate entities.
SUMMARY

The thesis is divided into 2 parts. Part I describes the establishment and management of units for rearing colostrum-free pigs in individual isolation from birth to 5 weeks of age. Thirteen of the 15 litters reared in this way were obtained by cesarean section. Two were born naturally, caught at birth, and placed in the units before they were able to suck. The survival rate was estimated at approximately 86%. The literature review discusses the transference of immunity from dam to offspring, the development of sow-milk replacers and the environmental requirements of newborn colostrum-free pigs, as well as the history, management and usefulness of isolation units.

Part II describes the experimental transmission of a previously unidentified encephalomyelitis of swine, which reached epidemic proportions in Ontario in 1958-59. The experimental pigs used were those reared in the isolation units. The causal agent, which was thought to be related to the virus of Teschen disease, was transmitted through 5 serial passages. It was shown that healthy pigs could contract the disease by direct contact with sick pigs, and that at least one mode of this transmission was by the oral and intranasal ingestion of feces from affected pigs. Attempts to transmit the disease using urine from affected pigs failed. Healthy pigs placed in air contact with affected pigs did not contract the disease.

The clinical signs, gross necropsy findings, hematology and bacteriology of experimental pigs are described, and the relationship of this disease with Teschen disease and with so-called "vomiting and wasting" disease is discussed.
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Table 1

AVERAGE VALUES FOR THE COMPOSITION OF SOW’S MILK

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<th>Total solids</th>
<th>Fat</th>
<th>Protein</th>
<th>Milk Sugar</th>
<th>Ash</th>
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Table 3

SURVIVAL RATES OF PIGS IN INDIVIDUAL ISOLATION

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<td>(A) No. of experimental treated value</td>
<td>(A) No. of experimental treated value</td>
<td>(A) No. of experimental treated value</td>
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<td>- -</td>
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<td>- -</td>
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<td>4*</td>
<td>12 8 8</td>
<td>4 3</td>
<td>- -</td>
</tr>
<tr>
<td>5</td>
<td>11 10 9</td>
<td>1 1</td>
<td>- -</td>
</tr>
<tr>
<td>6</td>
<td>14 9 8</td>
<td>1 0</td>
<td>4 2</td>
</tr>
<tr>
<td>7</td>
<td>6 4 4</td>
<td>- -</td>
<td>2 2</td>
</tr>
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<td>8</td>
<td>9 8 5</td>
<td>- -</td>
<td>1 1</td>
</tr>
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<td>9</td>
<td>8 6 6</td>
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<td>2 2</td>
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<td>10</td>
<td>10 10 10</td>
<td>- -</td>
<td>- -</td>
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<tr>
<td>11</td>
<td>10 6 6</td>
<td>1 1</td>
<td>3 2</td>
</tr>
<tr>
<td>12</td>
<td>7 6 6</td>
<td>- -</td>
<td>1 0</td>
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</tbody>
</table>

Total       121 86 76        16 14        19 14
% Survival       88        87        74
% Survival, overall 86

*Two litters were involved in experiment 4.
### Table 4

**Hemoglobin Values of Healthy Pigs and Pigs Affected with Viral Encephalomyelitis**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Healthy pigs</th>
<th>No. of pigs</th>
<th>Average values (g Hb)</th>
<th>Affected pigs</th>
<th>No. of pigs</th>
<th>Average values (g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7</td>
<td>8</td>
<td>6.85</td>
<td></td>
<td>12</td>
<td>8.97</td>
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<tr>
<td>8-14</td>
<td>8</td>
<td>8.79</td>
<td></td>
<td>4</td>
<td>8.62</td>
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<tr>
<td>15-21</td>
<td>6</td>
<td>10.13</td>
<td></td>
<td>1</td>
<td>8.8</td>
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</tr>
<tr>
<td>22-28</td>
<td>9</td>
<td>9.32</td>
<td></td>
<td>-</td>
<td>-</td>
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<tr>
<td>29-36</td>
<td>1</td>
<td>10.0</td>
<td></td>
<td>1</td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------</td>
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<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Growth</td>
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<tr>
<td>1. AIR SUPPLY</td>
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<td>3</td>
<td>0</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2. FEED SUPPLY</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>a) Fresh milk</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>5</td>
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<tr>
<td>b) Ration (after feeding)</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>4</td>
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</tr>
<tr>
<td>3. UNITS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Feed troughs</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>b) Units</td>
<td>50</td>
<td>18</td>
<td>32</td>
<td>-</td>
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<tr>
<td>4. CONGENITAL</td>
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<tr>
<td>a) Skin and ears</td>
<td>15</td>
<td>3</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>3</td>
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<tr>
<td>b) Rectums</td>
<td>57</td>
<td>3</td>
<td>54</td>
<td>-</td>
<td>-</td>
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<tr>
<td>c) Intestines</td>
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<td>1</td>
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<tr>
<td>5. NEONATAL</td>
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</tr>
<tr>
<td>a) Open plates</td>
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<td>10</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>b) Skin &amp; ears (Prior to entering units)</td>
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<td>2</td>
<td>4</td>
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<td>2</td>
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<tr>
<td>Route of inoculation</td>
<td>Exp. no.</td>
<td>No. of pigs</td>
<td>No. of brains</td>
<td></td>
<td></td>
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<td>----------------------</td>
<td>---------</td>
<td>-------------</td>
<td>---------------</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Treated</td>
<td>Showing typical clinical signs</td>
<td>Negative on bacterial culture</td>
<td>Showing typical microscopic lesions</td>
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<td>7</td>
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<td>0</td>
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<td>All route</td>
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<td></td>
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<tr>
<td>Total</td>
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<td>6</td>
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<tr>
<td>Total</td>
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<td>7</td>
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<tr>
<td>Inoculum</td>
<td>Route of inoculation</td>
<td>Exp. no.</td>
<td>No. of pigs</td>
<td>No. of brains</td>
<td></td>
<td></td>
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<td>-------------</td>
<td>---------------</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>showing typical treated clinical signs</td>
<td>negative on bacterial culture showing typical microscopic lesions</td>
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<td></td>
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<td>0</td>
<td>2</td>
<td>0</td>
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<tr>
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<td>oral and intranasal</td>
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<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>intracranial, intranasal and oral</td>
<td>7</td>
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<td>0</td>
<td>1</td>
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<tr>
<td></td>
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<tr>
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<td>intracranial, intranasal and oral</td>
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<td>0</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>(total)</td>
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<td>0</td>
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<td>7</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>oral and intranasal</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>0</td>
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<tr>
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<td>12</td>
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<td>(total)</td>
<td>9</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
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<td>direct</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
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<td>- contact</td>
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<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(total)</td>
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<td>2</td>
<td>4</td>
<td>2</td>
<td></td>
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<tr>
<td>Air contact</td>
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Table 8

CLINICAL SIGNS OF VIRAL ENCEPHALOMYELITIS
(Incubation Period and Duration of Illness)

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37 pigs infected by</td>
<td>13 pigs infected by oral</td>
</tr>
<tr>
<td></td>
<td>intracranial, oral and</td>
<td>and intranasal inoculation</td>
</tr>
<tr>
<td></td>
<td>intranasal inoculation</td>
<td>or by direct contact</td>
</tr>
<tr>
<td>Average</td>
<td>(hours)</td>
<td>(hours)</td>
</tr>
<tr>
<td>Range</td>
<td>(hours)</td>
<td>(hours)</td>
</tr>
<tr>
<td>Incubation period</td>
<td>57.5</td>
<td>94.5</td>
</tr>
<tr>
<td></td>
<td>48-100</td>
<td>68-144</td>
</tr>
<tr>
<td>Prodromal stage</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>6-48</td>
<td>1-72</td>
</tr>
<tr>
<td>Nervous stage</td>
<td>(15)*</td>
<td>(26)*</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Duration of illness</td>
<td>(35)*</td>
<td>(56)*</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*These figures are not fully representative because some of the pigs were killed in the terminal stages of the disease.
Table 9

TOTAL WHITE BLOOD CELL COUNTS OF HEALTHY PIGS AND PIGS AFFECTED WITH VIRAL ENCEPHALOMYELITIS

(i) Counts

<table>
<thead>
<tr>
<th></th>
<th>No. of samples</th>
<th>Average WBC/mm$^3$</th>
<th>S.Ds.</th>
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</thead>
<tbody>
<tr>
<td>Infected by &quot;all routes&quot;</td>
<td>14</td>
<td>9511</td>
<td>9853</td>
</tr>
<tr>
<td>Infected by oral and intranasal routes</td>
<td>9</td>
<td>14883</td>
<td>9693</td>
</tr>
<tr>
<td>Healthy pigs</td>
<td>12</td>
<td>6242</td>
<td>2422</td>
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</table>

(ii) Differences between counts

<table>
<thead>
<tr>
<th></th>
<th>Infected by &quot;all routes&quot;</th>
<th>Infected by oral and intranasal routes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected by oral and intranasal routes</td>
<td>5372 ±1166 (non sig.)</td>
<td>-</td>
</tr>
<tr>
<td>Healthy pigs</td>
<td>3269 ±2725 (non sig.)</td>
<td>8641 ±3372 (sig.)(P.05)*</td>
</tr>
</tbody>
</table>

*(Sig. P.05). There are fewer than 5 chances in 100 of a difference as large as the one obtained here being obtained by chance.
Table 10
BLOOD UREA NITROGEN ESTIMATIONS IN HEALTHY PIGS AND IN PIGS SUFFERING FROM ENCEPHALOMYELITIS

<table>
<thead>
<tr>
<th>Blood Urea Nitrogen Inoculation</th>
<th>Group A</th>
<th>Group B</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 pigs infected by intracranial, oral and intranasal inoculation</td>
<td>15 pigs infected by oral and intranasal inoculation or by direct contact</td>
<td>12 healthy pigs under 2 weeks of age</td>
<td></td>
</tr>
<tr>
<td>mg%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-30</td>
<td>11</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>30-60</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>60-100</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>100-125</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table 11
HEMATOLOGY

DIFFERENTIAL WHITE BLOOD CELL COUNTS OF HEALTHY PIGS AND PIGS AFFECTED WITH VIRAL ENCEPHALOMYELITIS

<table>
<thead>
<tr>
<th>Total W.B.C. per mm$^3$</th>
<th>Seg. %</th>
<th>Band %</th>
<th>Lymph %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affected pigs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(under 1 week of age)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6700</td>
<td>26</td>
<td>55</td>
<td>19</td>
</tr>
<tr>
<td>3200</td>
<td>68</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>6700</td>
<td>48</td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td>12250</td>
<td>38</td>
<td>43</td>
<td>19</td>
</tr>
<tr>
<td>6600</td>
<td>32</td>
<td>44</td>
<td>24</td>
</tr>
<tr>
<td>42000</td>
<td>36</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>14000</td>
<td>41</td>
<td>37</td>
<td>22</td>
</tr>
<tr>
<td>5600</td>
<td>41</td>
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<tr>
<td>6900</td>
<td>61</td>
<td>29</td>
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</tr>
<tr>
<td>3500</td>
<td>26</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>33000</td>
<td>13</td>
<td>27</td>
<td>60</td>
</tr>
</tbody>
</table>

| **Healthy pigs**       |        |        |         |
| (under 1 week of age)  |        |        |         |
| 6700                   | 38     | 12     | 49      |
| 4800                   | 22     | 22     | 56      |
| 4100                   | 28     | 16     | 56      |
| 4250                   | 29     | 5      | 66      |
| 16200                  | 26     | 42     | 32      |
Fig. 1. ISOLATION UNITS: PLAN
Circular shape, handles and hinged feed-pan trough cover.

Note:
2 handles to be opposite to each other and 28" from the floor.

Lay out of
ISOLATION-UNIT

Scale 1:10 (approx. 2m)
Fig. 2. ISOLATION UNITS: DETAIL.
Feed-pan (trough) and locking device.
Fig. 3. ISOLATION UNITS: FRONT ELEVATION.
Trough and locking device (false floor, support and cone).
Fig. 4. ISOLATION UNITS: (4) SECTION A-A.
Feed pan (trough), cover, false floor, support, pyramid (cone), air inlet and air outlet.
Fig. 5. ISOLATION UNITS: (5) COMPONENT PARTS.
Trough and gasket, cone and support, false floor and units.
Fig. 6. VENTILATION SYSTEM: DIAGRAM.
F.H.B. - Fan, heater and baffle plates.
B.H. - Booster heater.
T - Manual stop taps.
I.D. - Incoming-air ducts.
O.D. - Outgoing-air ducts.
1, 2, 3, 4, 5 - Units.
Fig. 7. ISOLATION UNITS LINKED UP TO THE VENTILATION SYSTEM. Insulated air ducts, manual stop-taps, rubber hoses, and plastic covers ballooned with air.
Fig. 8. ISOLATION UNITS LINKED UP TO THE VENTILATION SYSTEM.
Arrangement when 7 units are installed in 1 room.
Fig. 9. ISOLATION UNITS.
A healthy pig of 3-4 weeks of age feeding at the trough. (The plastic cover was temporarily removed for this photograph to be taken.)
Fig. 10. ARRANGEMENT OF ISOLATION UNITS IN EXP. 6.
Units 2 and 3, 4 and 5 in room 6, and units 1 and 2 in room 7 were linked up in pairs so that the 2nd unit of each pair received its air supply from the first. Initially 1 pig was placed in each unit. Pigs 223, 224 and 226 were infected, and at the onset of clinical signs, pigs 229, 230 and 231 were placed in with them.
Fig. 11. ARRANGEMENT OF ISOLATION UNITS.
When 7 units are installed in 1 room.
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Author: Thomas J. L. Alexander
Title: Establishment of Isolation Units, etc.
Code: CA2ON UF3 40AY2

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<td>Monographs</td>
<td>Acquisition, Boston Spa, West York, U.K.</td>
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THESIS:

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Alexander, Thomas J. L.

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The establishment of isolation units for newborn pigs.

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ALEXANDER, T. J. L.