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<td>Michael Santomas</td>
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MULBERRY HEART DISEASE OF SWINE

A Thesis
Presented to the Graduate School of the
University of Toronto in Partial Fulfillment of the
Requirements for the Degree of
Master of Veterinary Science

by
Ludwig William Geib

1959
Ludwig William Geib, was born in Brooklyn, New York, U.S.A. on January 3, 1934.

He received his primary education in the Brooklyn Public School System, and his secondary education at Newtown High School, Elmhurst, Queens, New York, U.S.A. In the fall of 1951 he entered the College of Agriculture, Cornell University, Ithaca, New York, U.S.A. In September of 1953 he enrolled in the College of Veterinary Medicine, Cornell University, Ithaca, New York, U.S.A. from which he received a D.V.M. in June 1957.

Studies toward the M.V.Sc. at the Ontario Veterinary College were begun in September 1957.
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To Dr. K. V. Jubb, thanks are extended for his advice and helpful suggestions.

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INTRODUCTION

The term "Mulberry Heart Disease" is applied to a condition of unknown etiology affecting swine. The disease is encountered most frequently in pigs in good physical condition being fattened for market. In Ontario it has been found in animals as young as four weeks of age and in animals as old as four years of age, but it occurs most often in the eight to twenty weeks of age group.

The condition has been described as Mulberry Heart Disease by Lamont et al. (22) in Ireland, Tutt and Gale (42), Vogt (43), and Wood (44) in England. Bennett (2,3) in the U.S.A. has described the lesions of Mulberry Heart Disease as a variation of Edema Disease of swine.

Mulberry Heart Disease is one of the major causes of losses in pigs of post-weaning age which are being fed for market. Of even more concern is our inability to make any satisfactory recommendations for the prevention of future outbreaks. Because of this, studies were undertaken to define the condition as it exists in Ontario with regard to specific lesions, epidemiology and bacteriology. Investigations were carried out in an attempt to determine the cause.
REVIEW OF THE LITERATURE

The first published reference to Mulberry Heart Disease was that of Lamont, Luke and Gordon (22) in Ireland who encountered the condition during routine post-mortem examinations. The history usually presented was that pigs from post-weaning up to 100 lbs. in weight had died suddenly with no premonitory signs. When premonitory signs were noted they consisted of episodes of acute respiratory distress. Generally only one or two pigs in a litter were affected. The lesions found on post mortem were: excess fluid in the pleural and peritoneal cavities, edema of the lungs, distention of the pericardial sac with serous fluid, and streaks or diffuse areas of hemorrhage under the epicardium and endocardium, usually confined to the right ventricle, but at times involving the left ventricle and the atria. These authors were of the opinion that the condition was a variation of Edema Disease or involved some type of anaphylactic reaction.

Timoney (39), Bennett (2,3), and Quin (31), in their descriptions of the gross pathological findings in pigs which they believed had died of Edema Disease, mentioned a few cases in which edema of the lungs and a distended pericardial sac containing serous fluid and clots of fibrin or a fluid which gelled on exposure to air. Hemorrhages in the myocardium and under the epicardium and
endocardium were stated to be occasional findings.

Goodwin (14) has described an outbreak of "Acute Circulatory Failure in a Herd of Pigs" on a farm where the early deaths were confined to pigs weighing 115-170 lbs. and subsequent mortality occurred in pigs weighing less than 100 lbs. Clinical signs noted were a slight yellow diarrhea, normal body temperature, dullness and muscular shivering. Some animals died suddenly without showing any premonitory signs. This author, in addition to recording post-mortem findings similar to those described by Lamont, Luke and Gordon (22), observed congestion of the parenchymatous organs, strands of fibrin among the viscera, and enlarged mesenteric lymph nodes. The outbreak of the condition in this herd of pigs was believed to have been precipitated by an increased feeding of fish meal in the ration, as deaths stopped when the fish meal was omitted from the diet. Goodwin was of the opinion that the increased amount of fish meal fed, "aggravated a pre-existing condition" and was not directly responsible for the disease. He drew attention to the similarity of the outbreak reported to Herztoe, Muscular Degeneration and Mulberry Heart Disease.

Tutt and Gale (42), Wood (44), and Vogt (43) have described their experiences with field outbreaks of Mulberry Heart Disease.

The cases described by Wood occurred in pigs from
ll weeks of age onwards. Clinical signs included anorexia, respiratory distress, subnormal body temperature, and shivering. Affected animals stood with their heads lowered and front legs spread apart as if trying to balance. When an affected pig was pushed on its side, it squealed loudly and exhibited paddling movements of its forelegs. Scouring was never observed, but some pigs were constipated. Gross lesions on post mortem were similar to those described by Goodwin (14). Wood suggested that the condition may be an enterotoxemia and drew an analogy to Clostridial enterotoxemia of lambs. He suggested that overfeeding may be a predisposing cause of Mulberry Heart Disease.

Vogt (43) suggested that Mulberry Heart Disease may be hereditary, in that the particular strains of pigs in the outbreaks he observed may not have been suitable for the "progressive management" under which they were reared.
THE DISEASE AS IT OCCURS IN ONTARIO

The data presented in this section are derived from 45 pigs which were submitted for routine necropsy examination at the Ontario Veterinary College from January 1958 to March 1959.

Materials and Methods

The cases were selected and designated as Mulberry Heart Disease on the following basis: a history of sudden death occurring in animals in a good state of nutrition; lesions consisting of peritoneal and pleural effusion, edema of the lungs, a distended pericardial sac containing an excess amount of fluid and strands of fibrin, and streaks of hemorrhage under the epicardium.

In each case a history was taken and a complete necropsy performed on the animal. Specimens of tissue were fixed in 10% formalin, and in addition in some cases heart muscle, skeletal muscle and eyes were fixed in Zenker's fluid.

Fixed tissues were routinely imbedded in paraffin and sections cut at approximately six microns and stained with Hematoxylin and Eosin. Selected paraffin sections were stained with Toluidine Blue for Nissl substance, Phosphotungastic acid-Hematoxylin for muscle striations, fibrin and glia, Holmes' Silver Nitrate (12) for axons, Luxol Fast Blue (25) for myelin, Periodic acid-Schiff for
basement membranes, and Kiton Fast Red (18) for muscle striations. In two cases frozen sections of brain were cut from formalin fixed tissue and stained with Oil Red O for neutral fat.

Aerobic cultures at 37° C. on sheep blood and MacConkey agar plates were made of the spleen (38 cases), lung (12 cases), pericardial fluid (eight cases), stomach (seven cases), lymph node (six cases), duodenum (16 cases), ileum (31 cases), jejunum (21 cases) and colon (20 cases). In addition the pericardial fluid was once cultured anaerobically at 37° C. on sheep blood agar, and twice smears were made and stained with Gram's stain. On two occasions the pericardial fluid was cultured at 37° C. in PPLO-broth enriched with horse serum. Segments of the intestine were also cultured anaerobically at 37° C. on sheep blood agar plates and smears made and stained with Gram's stain. The duodenum was cultured anaerobically in 16 cases, the ileum, jejunum and colon in 20 cases. Smears of the duodenum, ileum, jejunum and colon were made in 14 cases.

In seven cases the urine was examined for sugar.
Observations

Anamnesis

The history usually given was that pigs that were doing well and showing no previous signs of illness were found dead. When premonitory signs were noticed they consisted of: loss of appetite, dullness, ataxia, weakness in the hind legs and inability to rise. In some pigs that were moribund, rapid and labored respirations, opisthotonus, paddling movements of the limbs, swollen eyelids with protrusion of the eye (Plate 1) and intense scleral injection, and cyanotic discoloration of the ventral surfaces of the abdomen and thorax were noted. Body temperatures were generally within the normal range, but varied from a low of 96°F to a high of 105°F. The mortality rate varied between three and twenty-five percent. The age of the pigs affected ranged from four weeks to four years, with the majority of the cases occurring between eight and twenty weeks (Figure I). Fifty-two percent of the cases occurred in males, 48% in females. The disease has occurred during every month of the year (Figure II).

In most instances there was a change in the feeding practices from one to ten days prior to the onset of losses. Some animals had been weaned one to two weeks previously and were being fed a commercial "pig starter" ration. In other cases animals had recently been switched
Figure I

Ages of Pigs Affected by Mulberry Heart Disease

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**FIGURE II**

Monthly Incidence of Mulberry Heart Disease

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from a "starter" to a "grower" ration. In some of the cases occurring in young pigs a creep ration had been fed in addition to the sow's milk. Post-weaning cases occurred on farms both where the animals were self-fed a dry commercial ration and where they were slop-fed on a mixture of home grown grains and a commercial concentrate, but most cases occurred where animals were self-fed. Skim milk in a slop or in addition to the dry ration was being fed in a few outbreaks.

It is difficult to assess from the histories usually presented whether there was any scouring or constipation present in the affected animals. Many times the consistency of the feces had not been noted, or one was given the history that pigs in the pen had scoured or pigs had appeared to be constipated. On post mortem the majority of the pigs show feces of normal consistency.

Necropsy

Gross findings

The animal is usually in an excellent state of nutrition. In some cases a bluish discoloration of the ventral surface of the body and tips of the ears is noted. About 25% of the pigs necropsied show swollen eyelids and exophthalmos (Plate 1) with scleral injection.

On incision, the inguinal and parotid lymph nodes are enlarged, edematous, and sometimes hemorrhagic. In approximately 25% of the cases subcutaneous edema of the
eyelids, forehead, and abdomen is apparent.

The lesions in the thorax are characteristic. There is an excess (up to 250 ml.) of a clear or straw-colored fluid in the pleural cavity, and a markedly distended pericardial sac which displaces the lungs dorsally (Plate 2). The pericardial sac contains an excessive amount (up to 150 ml.) of a straw-colored fluid and clots of fibrin, or a fluid which gels on exposure to air (Plate 3, 4). The fibrin clots are soft, translucent, and usually weakly attached to the base of the heart. The pericardium is smooth and glistens. There is no blood in the pericardial fluid. The specific gravity of the fluid before it clots is 1.045. When the clots are removed, the specific gravity of the fluid is 1.010. The epicardium is streaked with linear ecchymotic hemorrhages (Plate 4). The hemorrhages are usually most severe in the right ventricle, but in many cases both ventricles and both atria are involved (Plate 5). Ecchymotic hemorrhages are found under the endocardium, with the papillary muscles and inter-ventricular septum as the most common sites. In severe cases the myocardium shows diffuse ecchymotic hemorrhages alternating with very pale areas (Plate 6). In some cases clotted blood is found in the ventricles; in others, all the blood is forced into the aorta and pulmonary artery.

The lungs are displaced by the distended pericardial sac, are congested and edematous and fail to collapse (Plate 2).
The edema involves the interlobular septae, the width of which may be thereby increased up to one cm. The pleura is generally wet and glistens, but in several cases there is a thin layer of fibrin, which is easily peeled away, covering the lungs. The mediastinum is not edematous. A white frothy mucoid material is found in the trachea and bronchi. On cut surface the lungs are edematous and exude a fluid which is a mixture of edema fluid and cyanotic blood. In three cases there is pneumonic consolidation of the tips of the anterior lobes of the lungs, but this is considered to be an incidental finding.

The abdominal cavity usually contains an increased amount (up to 200 ml.) of a clear or straw-colored fluid and thin, lace-like strands of fibrin among the intestines. The serosa of the intestine is congested. Many times the small intestine is almost empty, especially the anterior one-third, or its content is a mucoid, tan or green-colored material. The large intestine contains feces of normal consistency. There is no gelatinous edema of the mesentery of the coils of the colon, but these are wet and shining. In some cases the fundic portion of the gastric mucosa is reddened, but the mucous membrane is intact, and there is no hemorrhage into the lumen. The stomach is often filled with concentrate feed, but sometimes it is empty or contains abnormal material such as straw or wood shavings. There is no gelatinous edema of
the submucosa of the stomach.

The parenchymatous organs are congested. In approximately 30% of the cases a gelatinous edema of the submucosa of the gallbladder is noted (Plate 3). In these cases, the liver is severely congested, and its edges rounded.

The endocrine glands are normal. The kidneys are congested. On cut surface the renal parenchyma may bulge slightly. The medulla of the organ is generally congested. The capsule peels easily. With the exception of two male pigs in which edema of the tunica vaginalis is observed, the remainder of the urogenital organs are normal. In the seven cases where the urine was examined for sugar, it was negative in five cases and showed a trace in two.

The spleen is firm and intensely congested. The internal lymph nodes are enlarged, edematous, and at times hemorrhagic. The hemorrhage may only be found in the peripheral portions of the nodes, or throughout the substance of the nodes. The mediastinal lymph nodes are most often diffusely hemorrhagic.

In two animals there are lesions in the sub-lumbar muscles and diaphragm consisting of edema, pale streaks and hemorrhage.

The brains of two cases show swelling and partial herniation of the vermis of the cerebellum into the
foramen magnum. In all other cases the brain is normal or slightly wet in appearance. On section of fixed brains, in five cases there is observed bilateral and rather symmetrical areas of softening of the white matter in the frontal lobes. These are visible grossly as gray, translucent, depressed, discolored areas studded with small foci of hemorrhage (Plate 7, 8). The white matter of the gyri is affected most severely. In three of the five cases, the lesions involve the white matter of gyri in all lobes and the corpus medullare.

Microscopic findings

Heart  Focal hemorrhages are observed under the epicardium and endocardium; and tags of fibrin are attached to the epicardium. In some cases there is fibrin beneath the endocardium in these areas and the mesothelial cells are swollen and show occasional mitotic figures. The lymphatics are dilated and sometimes contain fibrin plugs. The myocardium beneath these foci is degenerate, the fibers have lost their cross striations and the myofibrils appear granular. Scattered among the myocardial fibers are diffuse areas of hemorrhage. In these areas the myocardial fibers are degenerate or necrotic. Areas of hemorrhagic and degenerate myocardium are most frequently found around blood vessels, or occupy the central area of a papillary muscle (Plate 9). The vessels show changes ranging from swelling of the endothelium (Plate 10)
and media (Plate 11) to necrosis of the vessel wall (Plate 12). Often hyaline thrombi are found in these vessels (Plate 13, 14). However, in many such areas, no thrombi could be found, and the vessels appear normal. The main branches of the coronary arteries are normal. Degeneration of the myocardial fibers in these areas is manifested by loss of cross striations with fragmentation of the myofibrils giving the fiber a granular appearance (Plate 15). In other areas there is severe fragmentation of the fibers with the fragments staining deeply with P.A.S. (Plate 16). Necrosis of fibers is evidenced by shrunken, dark-staining, hyaline sarcoplasm and dark-staining pyknotic nuclei. In some areas where the fibers are cut in cross section, there are foci in which the myofibrils are completely absent and only the sarcolemma remains (Plate 17).

Inflammatory cellular response to the degenerate and necrotic myocardial fibers is minimal or absent. A few mononuclear cells are sometimes found around the blood vessels or beneath the endocardium in these areas.

The distribution of the histological changes parallels those of the gross lesions in that the most severe changes are found in the areas of the heart noted to be affected grossly.

Lungs Severe congestion and edema is a consistent finding. The capillaries in the alveolar walls are
packed with erythrocytes, and the alveoli are filled with edema fluid. Occasionally there is hemorrhage into the alveoli. The apical and cardiac lobes of the lungs frequently show areas of compression atelectasis. The lobules are at times widely separated by edema fluid in the septae. Fibrinous tags, containing the occasional neutrophil or mononuclear cell, are adherent to the pleura. As mentioned in the gross findings, in three cases the anterior lobes of the lungs show pneumonic lesions. On microscopic examination the reaction is that of Virus Pneumonia of Pigs complicated by mild secondary bacterial infection.

Lymph nodes In many cases there is hemorrhage into the node, which is most severe in the peripheral portions. The lymphoid elements are separated by edematous fluid. In many instances eosinophils are present in increased numbers. They tend to accumulate in greatest numbers in the peripheral sinus of the node. None of the other cells of the myeloid series is observed.

Liver Congestion and edematous separation of the liver cords from the sinusoidal endothelium with atrophy of the liver cells (serous hepatitis) (Plate 18) are common findings. In several cases central lobular necrosis is present (Plate 19). In 13 cases marked edema of the wall of the gallbladder with complete lysis of the mucosa and muscular layer is observed. In such cases there
is complete loss of cellular elements, with only reticu-
ticular fibers remaining. This is a post-mortem change
duced by the action of bile on the tissues.

Kidney This organ generally shows congestion only.
However, in four cases hyaline eosinophilic material is
present around some of the arcuate arteries (Plate 20).
The adventitial cells of these vessels are swollen and
separated from one another. An apparent hyper-cellular-
ity of the glomeruli due to swelling and proliferation
of the epithelial cells is observed in a few cases (Plate 21),
and in these there is intercapillary edema which is shown
by the presence of an eosinophilic material between the
capillary endothelium and the visceral epithelium. In a
few cases there are mild degenerative changes in the tu-
bular epithelium, principally in the proximal convoluted
tubules with the formation of proteinaceous casts in the
lumen.

Intestine There are no significant lesions found
in most cases. When lesions are present they consist of
a mild catarrhal enteritis and infiltration of the mucosa
with mononuclear cells or eosinophilic leucocytes.

Eyes There is congestion of the uveal tract. In
one case, a proteinaceous exudate is present in the an-
terior chamber and there are focal areas of exudative
retinal detachment in the region of the optic papilla.
Beneath the areas of retinal detachment there is a hyaline
pink staining material which probably contains protein.
Adrenals Only two cases show any changes. In one case there is edema of the zona reticularis; in the other there is hemorrhage in the same zone.

Reproductive organs In the male specimens where edema of the tunica vaginalis was observed grossly, there are hemorrhages among the seminiferous tubules (Plate 22).

Skeletal muscle In two cases lesions are found in the skeletal musculature. The lesions are confined to the sub-lumbar muscles and diaphragm. The changes consist of hemorrhage, shrinkage, and fragmentation of fibers with the formation of retraction caps (Plate 23, 24). Degenerate fibers which are granular and have lost their cross striations are intermingled with others that appear normal.

Brain The brain lesions are arbitrarily divided into three developmental stages and an attempt is made to correlate them with the survival time of the animal after it has shown signs of illness. The brain was examined in 40 of the 45 cases described in this section.

Stage I The history is usually one of sudden death with no premonitory signs. In this stage either no brain lesions are apparent or vascular congestion and edema of the white matter are observed. Sixteen of the 40 brains examined are classified as Stage I.

Stage II The animals have usually been ill for a period of approximately 24 hours. In these instances
edematous separation of the fiber tracts in the white matter is usually found (Plate 25). This change is most obvious in the white matter of the frontal lobes. The nuclei of many of the oligodendroglia are swollen and others appear to be fragmented. Peri-neuronal and perivascular edema are apparent in the gray matter. Dark staining neurons are present in the upper layers of the cortex. Seventeen brains are classified as Stage II.

Stage III In these cases the animals usually show signs of illness for 24 hours or more. The usual finding is bilateral leucoencephalomalacia with foci of hemorrhage in the white matter of the rostral portions of the frontal lobes. However, focal areas of demyelination (Plate 26, 27, 28) are found throughout the white matter of the cerebrum. In three cases the white matter of all portions of the cerebrum is involved. Severe demyelination with axonal swelling and fragmentation (Plate 29, 30) characterizes the changes in these areas. Along the course of the fiber tracts, digestion chambers containing myelin and fragments of axon are observed. There are no macrophages or gitter cells present, and there is no gliosis. A stain for neutral fat on sections of the brain in two cases is negative. The vessels in the area show changes which vary from endothelial (Plate 31) and adventitial swelling and proliferation to necrosis of the vessel wall. In some vessels hyaline thrombi are
found (Plate 32). About other vessels there are perivascular cuffs. The cuffs are generally one to two cells in thickness and consist of mononuclear cells, including plasma cells and proliferated adventitial elements, although occasionally eosinophils and other neutrophils are found. When leucocytes are observed in cuffs, the eosinophil appears to be the predominant cell type (Plate 33).

The overlying gray matter shows much the same changes as in Stage II, except that they are more severe. In addition, endothelial proliferation and perivascular cuffing are observed in the vessels in the gray matter of a few specimens. These vascular changes and microscopic foci of softening are also occasionally found in the thalamus, brain stem and molecular layer of the cerebellum. Seven brains are classified as Stage III.

Spinal cord The spinal cord was examined in 15 cases. In 13 cases, no lesions are found. One case shows vacuolation of the neurons (Plate 34) and some demyelination of the spinal nerves. In another, proliferation of the endothelium in the vessels of the gray matter is noted.

No abnormalities, other than congestion, are found in the spleen, pancreas, pituitary gland, thyroid glands, parotid and mandibular salivary glands, semilunar ganglion and vagus nerve. The spleen may be markedly congested.

**Bacteriological findings**

Aerobic culture of the spleen, lung, liver, lymph
node and stomach were negative, that is, either no growth was obtained or organisms isolated were considered to be post-mortem invaders.

Smears of the pericardial fluid showed no visible organisms. Aerobic, anaerobic and PPLO cultures of the pericardial fluid were negative.

Smears of the four sections of intestine showed an almost equal number of cases with normal flora and cases with an excess of Gram-positive bacilli. Aerobic cultures of the intestine showed a preponderance of normal Escherichia coli. A hemolytic Escherichia coli was isolated from the duodenum once, from the ileum five times, from the jejunum once, and from the colon twice, but never in any one case from all segments of the intestine cultured. Anaerobic cultures of the various sections of intestine showed approximately equal numbers of cases from which were isolated Escherichia coli or large numbers of Clostridium perfringens.
DISCUSSION OF FINDINGS IN MULBERRY HEART DISEASE

The pathogenesis of the lesions of Mulberry Heart Disease are thought to involve a primary toxic injury to vascular endothelium, with secondary effects due to hypoxia from pulmonary edema and heart failure.

The results of injury to the vascular endothelium are apparent in the brain, heart, liver, kidney, and eyes where the early lesions are those of increased capillary permeability and loss of protein into the interstitial spaces. Degeneration and necrosis of the surrounding parenchyma occurs in these areas. In the brain the vascular changes include endothelial swelling and proliferation, adventitial proliferation, perivascular cuffing, hyaline thrombi, and necrosis of the vessel wall. The vessels in the heart show much the same changes as in the brain. The degenerate areas of myocardium are often in the center of a papillary muscle and many times surround an injured vessel. The serous hepatitis and edema of the gallbladder are manifestations of edema of the liver associated with an increased capillary permeability (17). The edema of the glomerular tufts and protein in the anterior chamber of the eye and under the detached areas of the retina are probably the result of injury to endothelium.

The contention that in addition to vascular injury
secondary effects may be due to hypoxia from pulmonary edema and heart failure finds support in the fact that pulmonary edema is a constant finding, and in some cases clotted blood is found in the heart. The finding of clotted blood in the left ventricle of the heart on necropsy is usually an indication of myocardial degeneration (28).

The severe demyelination observed in the brains of the seven cases of Mulberry Heart Disease classified as Stage III exhibit a similarity to changes described in "Moldy Corn Poisoning" of horses (4), and to lesions produced experimentally in the brain of monkeys (19), and rats (24) by the use of potassium cyanide. Lesions resembling those found in the brain in Stage II of Mulberry Heart Disease have been produced in pigs by the intramuscular injection of potassium cyanide (21). Potassium cyanide results in a histotoxic anoxia; that is, an interference with the activity of the intracellular oxidative enzymes (15). Experimental production of demyelinating lesions, other than by the use of potassium cyanide have been achieved in many ways (20), and still the mechanisms of demyelination remain enigmatical.

Meyer (15) has observed lesions of edema of the white matter and initial demyelination in a young boy with congestive heart failure and Cheyne-Stokes respiration. Here the lesions were probably due to anoxemia.

Greenfield (15) has observed the disappearance of
the oligodendroglia in some forms of diffuse sclerosis in man before any demyelination was evident. Analogous to this may be the swelling and fragmentation of the oligodendroglia described in the Stage II lesions in cases of Mulberry Heart Disease.

The absence of macrophages or glial reaction to the Stage III brain lesions of Mulberry Heart Disease is regarded as a reflection of the rapid progression of the condition to the death of the animal. The focal areas of softening in the gray matter may indeed be due to vascular injury, but it is unlikely that the lysis of the white matter is solely due to the changes in the blood vessels. Vasculitis and degenerative vascular changes of comparable type and severity are common in Salmonellosis and Erysipelas of swine, but these diseases are not attended by lysis of the white matter.

The author agrees with Wood (44) who states that overfeeding may be a predisposing cause of Mulberry Heart Disease. The basis for such an opinion is that the disease occurs most often in pigs on an above-average plane of nutrition, and the resemblance of the gross pathological changes of Mulberry Heart Disease to Enterotoxemia of lambs caused by Clostridium perfringens Type D. Brain lesions similar to those found in Mulberry Heart Disease have been observed by Hartley (16) in lambs which had died of Enterotoxemia. The author has observed brain
lesions in lambs which had died of Enterotoxemia, but
the lesions were more extensive than those described
by Hartley, with the white matter of the frontal lobes
showing severe malacia.

Vacuole formation in the neurons of the spinal cord
may be the result of post-mortem changes. The vacuoles
in the neurons of the spinal cord resemble those found
in sheep with Scrapie (45). Lindenberg (23) studied the
effects of hypoxia and anoxia in relation to the develop-
ment of various post-mortem changes in neurons. He found
that the duration of hypoxia preceding death determines
the type of post-mortem change in the neurons. The long-
er the period of hypoxia prior to death, the smaller the
degree of post-mortem change observed. The most severe
post-mortem cellular changes were found in cases of sud-
den death. Vacuole formation was not found where hypoxia
of 30 minutes or more preceded death.

The specific gravity of the pericardial fluid before
and after removal of the clot of fibrin indicates that the
increase in specific gravity was due to protein, mostly
fibrin, which probably came from the damaged vessels in
the heart and pericardium. Significantly however, there
is no cytological evidence of inflammation indicating that
this is not a pericarditis in the conventional sense.

An explanation for the rather selective involvement
of the right ventricle could be that its vascularization
is less efficient than is that of the left ventricle and interventricular septum (7). Thus, any interference in myocardial circulation would effect the right ventricle before the interventricular septum or left ventricle. In an interesting paper, Sporri (38) says that pigs may be predisposed to death by myocardial failure. By means of electrocardiographic studies he has shown that pigs, in contrast to other animals, have a very short diastolic interval. Due to compression of the vessels within the myocardium during systole, blood flow is almost entirely confined to the diastolic interval of the cardiac cycle. Tachycardia results in an even shorter diastolic interval. He also points out the fact that pigs have a low (0.3%) ratio of heart weight to body weight compared to the horse and dog, where the ratio is one percent.

The absence of an inflammatory cellular reaction to the hemorrhage and degeneration in the myocardium in cases of Mulberry Heart Disease may be due in part to a rapid onset and development of the lesions to a fatal termination. The hyaline thrombi are probably coagulated plasma.

Congestion and edema of the lungs are constant findings. This could account for the respiratory distress noted in some pigs before death. In the three cases showing areas of pneumonia, the lesions were not considered to be significant as they were limited in extent, and
many clinically normal pigs have been observed by the author to have slight pneumonic lesions. The atelectasis observed in some cases is probably the result of the pressure of the distended pericardial sac and excess pleural fluid on the lungs.

The finding of a few eosinophils in lymph nodes is normal (41). The large numbers which were at times observed in cases of Mulberry Heart Disease are believed to be a peculiarity of the pig and to have no significance for this disease.

The focal necrosis of the liver in some cases is regarded to be the consequence of the severe degree of central lobular congestion.

The hyaline eosinophilic material around some of the arcuate arteries in the kidney is probably coagulated plasma.

As previously mentioned, the gross pathological changes of Mulberry Heart Disease resemble those described for Enterotoxemia of lambs caused by Clostridium perfringens Type D. The finding of glucose in the urine of acute cases of ovine Enterotoxemia (27) prompted the testing of the urine for glucose in cases of Mulberry Heart Disease. Even though in five cases the urine was negative for sugar and showed only a trace in two, these findings are not regarded as significant since the animals from which the urine was obtained had been dead for some time, and much
of the glucose present would have been fermented.

The selection of cases was based on gross lesions previously described for Mulberry Heart Disease by Lamont et al. (22), Wood (44), and Goodwin (14). This rigid selection of cases does not include some cases where brain lesions, similar to those described as occurring in Mulberry Heart Disease, have been found in pigs with no, or minimal heart lesions. Generally, the brain lesions in these cases have shown reparative reactions such as gitter cells and glial proliferation. These cases may possibly have been "recovered" cases of Mulberry Heart Disease.

Concerning the variations in body temperature given in the anamnesis the sub-normal temperature may be explained on the basis of an in extremis hypothermia. The highest body temperature recorded (105° F.) is only 1.4° F. above the high normal for the pig of 103.6° F. as given by Dukes (10). This degree of temperature rise could have resulted from restraint of the pigs during the process of recording the temperature.

Even though only a small number of cases has been presented, it would appear from the data that the sex of the pig is not a factor to be considered, as 52% of the cases occurred in males and 48% in females.

From Figure II one might be tempted to conclude that there is a seasonal incidence of Mulberry Heart Disease, as most cases have occurred in June and November.
Such a conclusion would not be justified. In the number of cases tabulated in June, on two occasions owners had brought in two pigs for necropsy, and both animals were included in the monthly tabulation. In November one owner brought in three pigs for necropsy, and they were all included in the monthly tabulation. Often owners will bring in a single animal, even though other losses have occurred.

Regarding the statement that most cases occurred on farms where the pigs were self-fed, it must be remembered that the majority of the pigs that are being fed for market in Ontario are self-fed.

Only a small number of cases were examined bacteriologically, and these were not examined in a consistent manner. The pericardial fluid was not cultured more often as the gross pathological picture was not considered to be indicative of a bacterial pericarditis.

The isolation of a hemolytic *Escherichia coli* from a few cases of Mulberry Heart Disease has no significance. Campbell (6) has isolated it from normal pigs, sometimes in pure culture.

Gram-positive bacilli were often found on smear, and *Clostridium perfringens* often isolated from the intestine, but because these were inconstant findings they cannot be regarded as significant. Further work along these lines would appear to be of value as the gross pathological findings of Mulberry Heart Disease are very similar.
to those of Enterotoxemia in lambs caused by Clostridium perfringens Type D.

The history and necropsy findings of the following conditions may be confused with Mulberry Heart Disease.

"Moldy Corn Poisoning" in swine (34) often results in sudden deaths or signs of dullness and staggering before death. On necropsy hemorrhages are found in the body cavities. Microscopically there is necrosis of the liver. This condition can be differentiated from Mulberry Heart Disease in that there is a history of feeding "soft" corn. The hemorrhages consist of large extravasations of blood. Microscopically the myocardium is not affected, and no brain lesions have been reported.

Bennett (2, 3), Timoney (39), Lamont et al. (22), and Quin (31) have stated or suggested that Mulberry Heart Disease is a form of Edema Disease. This is not believed to be so, as no gelatinous edema of the coils of the colon or stomach wall has been found in Mulberry Heart Disease. Edema of the lungs in Edema Disease is reported as an occasional finding; it is a constant finding in Mulberry Heart Disease. No brain lesions have been reported in Edema Disease (9, 39), and we at the Ontario Veterinary College have not found any in approximately 50 consecutive cases examined, but they have been found in many cases of Mulberry Heart Disease. On bacteriological examination of the intestine in cases of Edema Disease a hemolytic
Escherichia coli, generally in pure culture, has almost always been isolated (40). This has not been true in cases of Mulberry Heart Disease in which we have found a hemolytic Escherichia coli in a minority of cases, but even in these the organism has not been in pure culture, nor in numbers which would suggest significance.

Vitamin E deficiency in swine (29) produces edema of the gallbladder, subcutaneous edema, liver lesions, lesions in heart and skeletal muscles, and effusion of serous fluid into the body cavities. It can be differentiated from Mulberry Heart Disease in that the muscle lesion is hyaline degeneration. In addition the common site for lesions in the skeletal muscle is the thigh musculature, rarely in the spinal muscles. Vitamin E deficiency also shows yellow fat, anemia and in many cases ulceration of the squamous mucosa of the stomach which are not found in Mulberry Heart Disease.

The occasional finding of eosinophils in perivascular cuffs in the brain in cases of Mulberry Heart Disease could be confused with those found in salt poisoning in swine (35). However, except for the presence of eosinophils the lesions bear no resemblance to those of Mulberry Heart Disease, the former being a polioencephalomalacia and the latter a leuкоencephalomalacia.

Herztod (13) is a sudden cardiac death of pigs. The necropsy lesions resemble those of Mulberry Heart Disease,
except that the cardiac and skeletal muscle lesions generally are described as resembling fish or chicken flesh. The muscles of the back, croup and axilla are most often involved. Microscopically the muscle lesions are characterized by albuminous, fatty, or in later stages hyaline degeneration with infiltration of round cells. Central lobular necrosis of the liver is also observed. No brain lesions have been described in Herztod. The muscle lesions in Mulberry Heart Disease are confined to the heart, sublumbar muscles and diaphragm, and are hemorrhagic and not hyaline in nature. Brain lesions are found in Mulberry Heart Disease.

Gossypol poisoning (36) produces subcutaneous edema, edema of the lungs, hydropericardium, congestion of the liver and edema of the gallbladder, pale muscles, and edematous lymph nodes. Microscopically the heart lesions are described as atrophy and cytoplasmolysis of fibers. The skeletal muscle lesions consist of atrophy and hypertrophy of the muscle fibers. No brain lesions have been described in Gossypol poisoning. In Gossypol poisoning the pigs usually show signs of illness for two to six days, and occasionally up to one month. The signs are dyspnea and progressive emaciation until the time of death. In Mulberry Heart Disease the heart muscle lesions are hemorrhagic and brain lesions are present. In Mulberry Heart Disease the pigs usually do not show any signs of illness,
or are only noted to be ill for a short time.

Anoxias, such as those produced by cyanide, rotenone and parathion may occasionally show lesions of edema of the lungs, hydropericardium, and hemorrhages on the heart involving the anterior descending groove of the coronary artery, and the hemorrhages are deep in the muscle rather than subepicardial (21); but the usual gross findings are characterized by a lack of lesions (30, 37). Clinical signs for the above poisonings include staggering, salivation, and respiratory distress. The history usually includes some reference to the source of the poison, such as recent spraying, dusting, etc. In Mulberry Heart Disease salivation has not been noted, and edema of the lungs and hydropericardium are constant findings. In addition, in Mulberry Heart Disease there are lesions in the liver, brain, and heart.

Poisoning with alpha napthyl thiourea (ANTU) (1) produces a cyanotic discoloration of the skin around the throat and base of the ears. There is a marked pulmonary edema and pleural effusion, as much as 1600 ml. However, there is no mention of heart or brain lesions, which are found in Mulberry Heart Disease.
MATERIALS AND METHODS-GENERAL

The mice used in Experiments 1, 2, 4, and 5 were mature Connaught strain Swiss white mice of both sexes. The mice were injected intravenously via the tail vein.

The pigs used will be described fully in the experiments in which they were utilized. Unless otherwise stated all intravenous injections in the pigs were made with an 18 gauge two inch needle and a 10, 20 or 50 ml. syringe into the jugular vein. The animals were restrained in dorsal recumbency on the ground or in a V-shaped wooden trough. The head was extended and the forelegs held flexed over the thorax.

Pericardial effusion-fluid from cases of Mulberry Heart Disease was obtained by introducing a sterile needle and syringe or a Pasteur pipette into the unopened pericardial sac. The point of entry was previously sterilized by searing the surface with a hot spatula or by pouring alcohol over the unopened pericardial sac and igniting it. The pericardial fluid thus obtained was cultured aerobically at 37°C on sheep blood and MacConkey agar plates and the remainder placed in sterile screw capped vials and stored at -25°C until needed.

The supernatant used in Experiments 3, 4, and 5 was prepared from the contents of the small intestine of pigs which had died of Mulberry Heart Disease. The
contents of the small intestine were milked into a beaker. A varying amount of saline solution was then added to the ingesta. The end result was a mixture of fluid consistency which was placed in the refrigerator (42°C) overnight. The next day the mixture was filtered through two thicknesses of gauze. The filtrate was then centrifuged at 3000 r.p.m. for 30 minutes and the supernatant removed and stored at -25°C.

In Experiment 1 blood samples were taken from the pigs just prior to the administration of Clostridium perfringens Type D toxin and again 14 days later. The samples were allowed to clot and then centrifuged at 3000 r.p.m. for 15 minutes. The serum was withdrawn with a Pasteur pipette and placed in rubber stoppered tubes and stored at -25°C, until used.

The freeze-dried Clostridium perfringens Type D toxin * was stored at -25°C, until just prior to use, at which time it was reconstituted with 20 ml. of broth infusion media.

* Obtained from Burroughs Wellcome Ltd, Beckenham, Kent.
EXPERIMENT 1

Effect of Clostridium perfringens Type D Toxin in Pigs

The resemblance of the brain lesions of Mulberry Heart Disease to those described by Hartley (16) in lambs which had died of Enterotoxemia due to Clostridium perfringens Type D, and the finding of large numbers of organisms morphologically resembling Clostridia on smears made from the intestine of pigs which died of Mulberry Heart Disease prompted the attempt to reproduce the disease by the administration of Clostridium perfringens Type D toxin to pigs.

Materials and Methods

The freeze-dried Clostridium perfringens Type D toxin was reconstituted and dilutions in saline were made. The toxin was diluted so that 0.25 ml. of each dilution contained $6 \times 10^{-3}$, $6 \times 10^{-4}$, $6 \times 10^{-5}$, and $3 \times 10^{-5}$ ml. of the reconstituted toxin. One-quarter ml. of each of the above dilutions were injected intravenously into pairs of mice to determine the mouse MLD of the toxin, and the mice were then observed for a period of 60 hours.

Reference to Table I reveals that the mouse MLD of the toxin was between $3 \times 10^{-5}$ and $6 \times 10^{-5}$ ml. of the reconstituted toxin.
Six, nine week old Yorkshire pigs (140N, 141N, 142N, 144N, 146N, 149N) weighing between 55 and 60 lbs. were given the reconstituted *Clostridium perfringens* Type D toxin intravenously. Four pigs (140N, 141N, 142N, 144N) were each given 1.5 ml. of toxin and two pigs (146N, 149N) were each given six ml. of the toxin.

The four pigs that were given 1.5 ml. of toxin received between 25,000 and 50,000 mouse MLD doses. The two pigs that were given six ml. of toxin received between 100,000 and 200,000 mouse MLD doses.

The rectal temperature of the pigs and a blood sample were taken just prior to the injection of the toxin. Temperatures were again taken five minutes and one hour after injection. The animals were observed again six hours after injection and once daily for the next two days.
**TABLE I**

**Determination of the Mouse MLD of Clostridium perfringens Type D Toxin**

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Amount of Toxin</th>
<th>Survival Time *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6x10^{-3} ml.</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>6x10^{-3} ml.</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>6x10^{-4} ml.</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>6x10^{-4} ml.</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>6x10^{-5} ml.</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>6x10^{-5} ml.</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>3x10^{-5} ml.</td>
<td>survived</td>
</tr>
<tr>
<td>8</td>
<td>3x10^{-5} ml.</td>
<td>survived</td>
</tr>
</tbody>
</table>

* Expressed in hours post-injection.
Results

Following the administration of the toxin all pigs showed similar effects. The effects noted immediately after injection were rapid shallow abdominal respiration, and transient hyperemia of the skin. Most of the animals became recumbent within three minutes. The postures assumed were those of lateral recumbency, sternal recumbency, and a dog-sitting position. The pigs generally kept their eyes closed.

Within 15 minutes after injection an improvement in the condition of the animals was noted. The improvement was evidenced by a lowered rate of respiration, and some of the pigs got up and moved about. There was no noticeable difference in the behavior of the pigs which received 1.5 ml. and those which received six ml. of the reconstituted toxin.

In regard to post-injection temperatures as compared with pre-injection temperatures (Table II), the only animal which showed an increased temperature five minutes after injection was pig 140N whose temperature at the time was 104.7°F. The remainder of the animals showed a slightly lower (0.1 to 0.5°F.) temperatures. Within an hour the temperature of all the pigs was within the range of normal as given by Dukes (10) of 101.6 to 103.6°F.

When the animals were again observed six hours
after injection they showed no abnormal signs. The pigs exhibited normal behavior at all subsequent observations.
TABLE II

Effect of Clostridium perfringens
Type D Toxin in Pigs

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>1h0N</th>
<th>1h1N</th>
<th>1h2N</th>
<th>1h3N</th>
<th>1h4N</th>
<th>1h5N</th>
<th>1h6N</th>
<th>1h7N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ml. of toxin</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mouse MLDs (25,000-50,000) (100,000-200,000)

Pre-injection temperature °F: 104.2 104.6 103.4 103.8 103.7 103.4

Temperature °F:
5 minutes post-injection: 104.7 104.1 103.1 103.6 103.3 103.0

1 hour post-injection: 103.0 103.5 103.5 103.1 102.5 102.8

Result: all animals survived

° Temperatures are expressed in degrees Fahrenheit.
EXPERIMENT 2

Examination of Pig Serum
for Antibodies Against
Clostridium perfringens Type D Toxin

From the results of the previous experiment it appeared that pigs are not very susceptible to Clostridium perfringens Type D toxin. Therefore it was decided to examine the sera of the pigs used in Experiment 1 to determine if they contained or had developed specific antibodies in response to the injected Clostridium perfringens Type D toxin.

Materials and Methods

Prior to, and 14 days after the administration of Clostridium perfringens Type D toxin to the pigs in Experiment 1, blood samples were taken and the serum removed. In the present experiment each sample of serum was examined as outlined below for its ability to protect mice against Clostridium perfringens Type D toxin.

Both lots of sera, various dilutions of toxin, and mixtures of serum and toxin were injected into groups of two mice. The tests run on the two lots of sera were done at different times. The potency of the toxin was determined just prior to each test in order to assess the degree of deterioration, if any, which occurred on storage. The toxin was stored at \(-25^\circ C\) in the
interval between tests.

The tests were set up as follows: 0.25 ml. of each of the 12 serum samples was injected into groups of two mice to determine if the serum alone was toxic.

To confirm the potency of the toxin, it was injected into pairs of mice. The toxin was diluted with saline so that 0.25 ml. of each dilution contained $6 \times 10^{-3}$, $6 \times 10^{-4}$, $6 \times 10^{-5}$, or $3 \times 10^{-5}$ ml. of the reconstituted toxin.

To assess the potency of the toxin, in the tests performed on the sera obtained prior to the injection of toxin in pigs in Experiment 1, three pairs of mice were injected with the $6 \times 10^{-4}$, $6 \times 10^{-5}$ and $3 \times 10^{-5}$ dilutions of the toxin. To ascertain the potency of the toxin, in the tests performed on the sera obtained 1½ days after the administration of toxin to pigs in Experiment 1, three pairs of mice were injected with the $6 \times 10^{-3}$, $6 \times 10^{-4}$, and $6 \times 10^{-5}$ dilutions of toxin.

In order to evaluate the protective function of the pig serum, 36 pairs of mice were given a mixture of serum and toxin. Each mouse received 0.5 ml. of the mixture. Three dilutions of toxin ($25 \times 10^{-3}$, $25 \times 10^{-4}$ and $25 \times 10^{-5}$) were tested against both samples of the serum of each pig. One ml. of each of the three dilutions of toxin was mixed with one ml. of serum and allowed to stand at room temperature for 30 minutes. Each mixture was then injected into groups of two mice. Therefore,
the mice received approximately the following amounts of the reconstituted toxin; $6 \times 10^{-3}$, $6 \times 10^{-4}$, $6 \times 10^{-5}$ ml.

In the tests on the sera obtained prior to the injection of toxin in pigs in Experiment 1, the mice were observed for 70 hours. The mice, in the tests on the sera obtained 14 days after the injection of toxin to pigs in Experiment 1, were observed for 50 hours.
Results

Serum obtained from pigs in Experiment 1 prior to the injection of *Clostridium perfringens* Type D toxin:

All of the mice which had been given 0.25 ml. of the various sera alone, survived.

By referring to Table III, part A, it can be seen that the $6 \times 10^{-4}$ and $6 \times 10^{-5}$ dilutions of toxin were lethal to mice; although only one of the mice which were given the $6 \times 10^{-5}$ dilution of the toxin died. The $3 \times 10^{-5}$ dilution of toxin was not lethal to mice.

Reference to Table IV, parts A1 and A2 will show that all the mice which received the $6 \times 10^{-3}$ and $6 \times 10^{-4}$ dilutions of toxin in the mixture of serum and toxin injected died. Of the mice receiving the $6 \times 10^{-5}$ dilution of toxin in the mixture of serum and toxin, both mice receiving the serum sample from pig 142N died. One of the pair of mice that received serum samples from pigs 140N, 144N, and 146N died and the other mouse survived. Both of the pairs of mice which received the serum samples from pigs 141N and 149N survived.

Serum obtained from pigs in Experiment 1 fourteen days after the injection of *Clostridium perfringens* Type D toxin:

All of the mice which had been given 0.25 ml. of the various sera only, survived.

Reference to Table III, part B, will show that the $6 \times 10^{-3}$, $6 \times 10^{-4}$, and $6 \times 10^{-5}$ dilutions of toxin were lethal
to all mice.

By referring to Table IV, parts $B_1$ and $B_2$ it can be seen that all mice injected with the $6 \times 10^{-3}$ dilution of toxin in the mixture of serum and toxin died. The mice which received the $6 \times 10^{-4}$ dilution of toxin in the mixture of serum and toxin died with the exception of one of the mice which had received the serum sample from pig $149N$. Of the mice receiving the $6 \times 10^{-5}$ dilution of toxin in the mixture of serum and toxin, one of the pair of mice that received serum samples from pigs $140N$, $142N$, $144N$, $146N$ and $149N$ died and the other mouse survived. Both of the pairs of mice which received the serum sample from pig $141N$ survived.
TABLE III

Effect of Clostridium perfringens
Type D Toxin in Mice

Part A

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Toxin Dilution</th>
<th>Amount</th>
<th>Survival Time *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$6 \times 10^{-4}$</td>
<td>0.25 ml.</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>$6 \times 10^{-4}$</td>
<td>0.25 ml.</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>$6 \times 10^{-5}$</td>
<td>0.25 ml.</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>$6 \times 10^{-5}$</td>
<td>0.25 ml.</td>
<td>survived</td>
</tr>
<tr>
<td>5</td>
<td>$3 \times 10^{-5}$</td>
<td>0.25 ml.</td>
<td>survived</td>
</tr>
<tr>
<td>6</td>
<td>$3 \times 10^{-5}$</td>
<td>0.25 ml.</td>
<td>survived</td>
</tr>
</tbody>
</table>

* Expressed in hours after injection.
TABLE III (continued)

Part B

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Toxin Dilution</th>
<th>Amount</th>
<th>Survival Time *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$6 \times 10^{-3}$</td>
<td>0.25 ml.</td>
<td>0.16</td>
</tr>
<tr>
<td>2</td>
<td>$6 \times 10^{-3}$</td>
<td>0.25 ml.</td>
<td>0.75</td>
</tr>
<tr>
<td>3</td>
<td>$6 \times 10^{-4}$</td>
<td>0.25 ml.</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>$6 \times 10^{-4}$</td>
<td>0.25 ml.</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>$6 \times 10^{-5}$</td>
<td>0.25 ml.</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>$6 \times 10^{-5}$</td>
<td>0.25 ml.</td>
<td>15</td>
</tr>
</tbody>
</table>

* Expressed in hours after injection.
TABLE IV

Serum Neutralization Tests on
Clostridium perfringens Type D Toxin
by Injection in Mice

Part A

Method

<table>
<thead>
<tr>
<th>Mouse</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ml. serum</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Toxin dilution</td>
<td>$6 \times 10^{-3}$</td>
<td>$6 \times 10^{-3}$</td>
<td>$6 \times 10^{-4}$</td>
<td>$6 \times 10^{-4}$</td>
<td>$6 \times 10^{-5}$</td>
<td>$6 \times 10^{-5}$</td>
</tr>
<tr>
<td>Ml. toxin</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Mouse MLDs</td>
<td>100</td>
<td>100</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
### TABLE IV (continued)

#### Part A2

**Survival Time** *

<table>
<thead>
<tr>
<th>Mouse</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1hON</td>
<td>0.13</td>
<td>0.13</td>
<td>1</td>
<td>1h</td>
<td>4</td>
<td>survived</td>
</tr>
<tr>
<td>Sample 1h1N</td>
<td>0.13</td>
<td>0.13</td>
<td>1</td>
<td>1.5 (survived)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1h2N</td>
<td>0.13</td>
<td>0.13</td>
<td>0.2</td>
<td>0.2</td>
<td>1.5</td>
<td>1h</td>
</tr>
<tr>
<td>Sample 1h4N</td>
<td>0.13</td>
<td>0.13</td>
<td>0.2</td>
<td>1h</td>
<td>7</td>
<td>survived</td>
</tr>
<tr>
<td>Sample 1h6N</td>
<td>0.13</td>
<td>0.13</td>
<td>0.5</td>
<td>1.2</td>
<td>4</td>
<td>survived</td>
</tr>
<tr>
<td>Sample 1h9N</td>
<td>0.13</td>
<td>0.13</td>
<td>1</td>
<td>1.1 (survived)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Expressed in hours after injection.
TABLE IV (continued)

**Part B**

**Method**

<table>
<thead>
<tr>
<th>Mouse</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Ml. serum</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Toxin dilution</td>
<td>6x10^{-3}</td>
<td>6x10^{-3}</td>
<td>6x10^{-4}</td>
<td>6x10^{-4}</td>
<td>6x10^{-5}</td>
<td>6x10^{-5}</td>
</tr>
<tr>
<td>Ml. toxin</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Mouse MLDs</td>
<td>100</td>
<td>100</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
TABLE IV (continued)

Part B2

Survival Time *

<table>
<thead>
<tr>
<th>Mouse</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Survived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 140N</td>
<td>0.16</td>
<td>1.5</td>
<td>5.5</td>
<td>5.5</td>
<td>1.5</td>
<td>survived</td>
<td></td>
</tr>
<tr>
<td>Sample 141N</td>
<td>0.08</td>
<td>1</td>
<td>4.5</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 142N</td>
<td>0.25</td>
<td>0.5</td>
<td>1.25</td>
<td>7.5</td>
<td>3</td>
<td>survived</td>
<td></td>
</tr>
<tr>
<td>Sample 144N</td>
<td>0.5</td>
<td>0.5</td>
<td>1.3</td>
<td>3</td>
<td>15</td>
<td>survived</td>
<td></td>
</tr>
<tr>
<td>Sample 146N</td>
<td>0.8</td>
<td>0.25</td>
<td>2</td>
<td>5.5</td>
<td>2</td>
<td>survived</td>
<td></td>
</tr>
<tr>
<td>Sample 149N</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>7 survived</td>
</tr>
</tbody>
</table>

* Expressed in hours after injection.
EXPERIMENT 2

Effect of Intestinal Contents and Intestinal Supernatants in Pigs

An attempt was made to reproduce Mulberry Heart Disease by the oral administration of intestinal contents, and by intravenous injection of intestinal supernatants from pigs which had died of the disease. The resemblance of this condition to Edema Disease and the success achieved by Timoney (40) in reproducing it with intestinal supernatants prompted this experiment.

Materials and Methods

A six week old Yorkshire pig (3A) weighing approximately 25-30 lbs. was given via a stomach tube the contents of the small intestine of a pig which had died of Mulberry Heart Disease. The ingesta was mixed with water and a total volume of 200 ml. was administered.

Intravenous injections of intestinal supernatants prepared in the manner previously described, from pigs which had died of Mulberry Heart Disease were given to four pigs (140N, 143N, 146N, 148N) at different times. In all cases, temperatures were recorded at intervals and clinical signs noted.

Pig 140N, a 12 week old Yorkshire weighing approximately 90-95 lbs., previously used in Experiment 1, was
given 20 ml. of an intestinal supernatant. The injection
time was approximately one minute. Three days later the
same animal was given another 10 ml. of the same intesti-
nal supernatant. The supernatant had been stored at
-25° C. for the three day interval. Three days after
the second injection of supernatant the animal was killed
by bleeding.

Pig 146N, a 14 week old Yorkshire weighing approxi-
ately 100-110 lbs., previously used in Experiment 1,
was given 50 ml. of an intestinal supernatant. The in-
jection time was approximately one minute.

Pig 143N, a 14 week old Yorkshire weighing approxi-
ately 100-110 lbs., was given 75 ml. of an intestinal
supernatant. The injection time was approximately two
minutes.

Pig 148N, a 14 week old Yorkshire weighing approxi-
ately 100-110 lbs., was given 95 ml. of an intestinal
supernatant. The injection time was approximately two
minutes. On the fourth day after injection the animal
was killed by electrocution.

Tissues of pigs 140N and 148N which were examined
microscopically, were fixed in 10% formalin, with the
exception of the eyes of pig 148N which were fixed in
Zenkers fluid. All tissues were imbedded in paraffin
and sections cut at six microns. All sections were
stained routinely with Hematoxylin and Eosin. Selected
sections from pig 148N were stained with Holmes' Silver Nitrate (12) for axons, Luxol Fast Blue (25) for myelin, Toluidine Blue for Nissl substance, and Periodic acid-Schiff for basement membranes.
Results

Pig 3A which received the intestinal contents orally never exhibited any abnormal signs. The animal was observed every two hours for six hours after dosing and once daily for one week.

Pig 140N

Immediately after injection the animal showed a transient hyperemia of the skin and open mouthed breathing. Its temperature at this time was 105.2°F, heart rate 140. Within two to three minutes the animal vomited, and then retched for 10 minutes. The pig would lie down, but would soon rise to a dog sitting position and retch. At 15 minutes the animal was lying on its side and still showed labored respirations, but not to the degree observed immediately after injection. Temperature at this time was 105.6°F, heart rate 156. At 30 minutes the animal would get up and walk away if disturbed. Respirations at this time were normal, temperature 105.6°F. At two hours the temperature of the pig was 106.8°F. It preferred to lie down, but would get up when disturbed. At 18 hours its temperature was 105.0°F. It had eaten some food, but still preferred to lie down. At 24 hours its temperature was 103.8°F, the pig appeared normal and had eaten well.

Three days later the animal was given another 10 ml. of the same intestinal supernatant. Immediately
after injection it exhibited a rapid rate of respiration and hyperemia of the skin. Within five minutes the hyperemia of the skin had disappeared and the rate of respiration was almost normal. The animal preferred to lie down, but could easily be aroused. At ten minutes it was up and nosing about the bedding. At 15 minutes it began to shiver. This lasted for 15 minutes, after which the animal appeared normal.

Three days after the second injection of intestinal supernatant the animal was killed by bleeding.

Necropsy

Gross findings

The pericardial sac and heart are normal, and aside from an eight cm. in length necrotic area about the jugular vein which extends into the neighboring muscle, no other lesions are observed.

Microscopic findings

Microscopic lesions, with the exception of the jugular phlebitis and suppuration and necrosis in the surrounding muscle, are confined to the central nervous system. The lesions consist of neuronal degeneration observed in all parts of the central nervous system examined. This is manifested by dark staining neurons with shrunken cytoplasm and pyknotic nuclei. Spinal cord- increase in the number of glial elements in the gray matter, with some focal
aggregations of glia. The glial groups are all adjacent to neurons (satellitosis).

Medulla- proliferation of the adventitia of some vessels.

Thalamus- proliferation of the adventitia of the vessels.

Hippocampus- swelling and proliferation of endothelium, hemorrhages into the perivascular space.

Frontal lobes- apparent mild, diffuse increase in the number of glia in the white matter. Swelling of endothelial cells and proliferation of the adventitia of the vessels in both white and gray matter.

**Bacteriological findings**

Aerobic cultures at 37° C. of spleen and mediastinal lymph node on sheep blood and MacConkey agar plates were negative. Culture of the necrotic material about the jugular vein yielded a mucoid Escherichia coli and *Corynebacterium pyogenes*.

**Fig 143N**

The pre-injection temperature of this animal was 103.8° F. Hyperemia of the skin and injection of the sclera of the eye noted immediately after injection, disappeared within three minutes. The animal stood with its head lowered and was reluctant to move. At 15 minutes its temperature was 105.2° F., and it passed a soft stool with a large amount of clear mucus which contained flecks of blood. At 20 minutes the animal began to move about,
but still appeared slightly depressed. At 18 hours, it had eaten, was alert, and had a temperature of 102.5° F. At 42 hours the pig showed no abnormal signs, and its temperature was 103.0° F.

Fig 116N

The pre-injection temperature of the animal was 105.2° F. Injection of the scleral vessels and hyperemia of the skin were noted immediately after injection. These disappeared within two minutes. At this time the pig was reluctant to move and stood in one position with its head down and tail straight. At five minutes the animal was more alert and its tail was curled. At 15 minutes its temperature was 106.0° F., it began to froth at the mouth, and 15 minutes later vomited. After this, the animal laid down and would stay down unless disturbed. At 18 hours the temperature of the pig was 104.2° F., it was up and alert, and had eaten. At 42 hours its temperature was 103.8° F., and it showed no abnormal behavior.

Fig 118N

The pre-injection temperature of the pig was 103.8° F. Immediately after injection it staggered and went down. The upper eyelids were swollen and the pupils of the eyes were dilated. Respiration ceased for about 15 seconds, and transient cyanosis developed. The swollen eyelids and cyanosis passed off rapidly. When the pig attempted to rise, it could only get to a dog sitting position with
the front limbs knuckled over at the carpus. It showed a flaccid posterior paralysis and was insensitive to needle pricks about the tail and hindquarters. Ten minutes later the pig was noted to have some sensation about the tail. At this time the animal had a temperature of 104.8° F. and it began frothing at the mouth and vomited. Respiration was rapid and labored (120 per minute). It appeared to be blind, since there was no reaction to a finger thrust toward the eye. The respiration gradually returned to normal. At 30 minutes it got up and walked with a wobbly and incoordinated gait and the animal appeared to see again, as it reacted to a finger thrust toward the eye. At 18 hours the pig had a temperature of 102.8° F. It could get up, but was wobbly on all fours. At 42 hours its temperature was 104.4° F. At this time it appeared to have lost weight, had a dry scaly skin and preferred to lie down. When forced to get up, the animal was incoordinated, especially in the hindquarters. At 66 hours it was still down, but could be forced to stand. Its temperature at this time was 105.0° F. One hundred hours after injection of the supernatant the pig was killed by electrocution.

Necropsy

Gross findings

The lungs show subpleural petechial hemorrhages. Subcapsular ecchymotic hemorrhages and ascarid scars are
evident in the liver. The pericardial sac and heart are normal. Numerous infarcts are found in both kidneys. The remainder of the parenchymatous and urogenital organs are normal. The stomach is full of a cereal type feed, and has a gray colored mucoid material adherent to the mucosa in the region of the cardia. The joints of the left hind leg are enlarged and contain excess fluid. The brain and spinal cord are normal.

Microscopic findings

The lesions found are:

Kidney- infarcts, with infiltration of mononuclear cells into the necrotic parenchyma.

Heart- in the wall of the right ventricle degenerating and necrotic myocardial fibers are being replaced by fibroblasts.

Spinal cord- neuronal satellitosis.

Brain stem- a small focus of lysis of white matter with infiltration of glia (early glial nodule).

Thalamus- neuronal degeneration, as manifested by dark staining neurons with shrunken cytoplasm and pyknotic nuclei.

Frontal lobes- neuronal degeneration similar to that in the thalamus.

Bacteriological findings

Aerobic cultures at 37° C. of spleen, joint fluid, kidney, liver, lung, and cerebrospinal fluid on sheep
blood and MacConkey agar plates were negative. Culture of the stomach yielded a mucoid *Escherichia coli*, culture of the intestine yielded a normal *Escherichia coli*. 
EXPERIMENT 4.

Toxicity to Mice of Intestinal Supernatants from Cases of Mulberry Heart Disease

In order to quickly screen for toxicity a number of intestinal supernatant samples, it was decided to use mice as experimental animals.

Materials and Methods

The intestinal supernatants which had been frozen were allowed to thaw at room temperature. They were mixed by inverting the vial several times, and then injected into mice. The remaining intestinal supernatant was refrozen.

A total of four mice were used for each sample. Two mice were given 0.25 ml. and two mice given 0.5 ml. of the intestinal supernatant. Tests were concluded at 36 or 48 hours after injection of the supernatant.
Results

Table V shows that only two samples of intestinal supernatant, 892 PM and 2088 PM resulted in deaths in mice, and then only when 0.5 ml. was injected.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount Injected</th>
<th>Survival Time *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1300 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td>892 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>0.03 and 13.5</td>
</tr>
<tr>
<td>1376 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td>1718 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td>1759 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td>1857 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
</tbody>
</table>

* Expressed in hours post-injection.
### TABLE V (continued)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount Injected</th>
<th>Survival Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>4805A PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td>4805B PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td>1278 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td>2089 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td>2088 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>0.5 and 0.91</td>
</tr>
<tr>
<td>2118 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td>2141 PM</td>
<td>0.25 ml.</td>
<td>both survived</td>
</tr>
<tr>
<td></td>
<td>0.5 ml.</td>
<td>both survived</td>
</tr>
</tbody>
</table>

* Expressed in hours post-injection.
EXPERIMENT 5

Serum Neutralization Tests on Lethal Intestinal Supernatants

In Experiment 4, two samples of intestinal supernatant were found to be lethal to mice when 0.5 ml. was injected intravenously. A serum neutralization test using antisera of Clostridium perfringens Types A, B, C, D and E were run against the two lethal samples of intestinal supernatant.

Materials and Methods

The intestinal supernatants used, 892 PM and 2088 PM were those found to be lethal to mice in Experiment 4.

For each serum neutralization test 12 mice were used. One and one-half ml. of the intestinal supernatant was mixed with 0.5 ml. of the antisera of Clostridium perfringens Types A, B, C, D, E and allowed to stand at room temperature for 30 minutes. Groups of two mice were then injected with 0.65 ml. of the mixture of each type. Two mice were used to confirm the lethal effect of each intestinal supernatant and received 0.5 ml. intravenously.

* Obtained from Burroughs Wellcome Ltd., Beckenham, Kent.
Results

Reference to Table VI will show that 0.5 ml. of intestinal supernatants 892 PM and 2088 PM were still lethal to mice.

The antisera of *Clostridium perfringens* Types A, B, C, D, E failed to protect mice against the lethal effects of intestinal supernatants 892 PM and 2088 PM (Table VII).
**TABLE VI**

Effect of Intestinal Supernatants

892 PM and 2088 PM in Mice

<table>
<thead>
<tr>
<th>Supernatant</th>
<th>mL. Supernatant</th>
<th>Mouse</th>
<th>Survival Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>892 PM</td>
<td>0.5</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>892 PM</td>
<td>0.5</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>2088 PM</td>
<td>0.5</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>2088 PM</td>
<td>0.5</td>
<td>2</td>
<td>1.16</td>
</tr>
</tbody>
</table>

* Expressed in hours after injection.
TABLE VII

Neutralization in Mice of Lethal Intestinal Supernatants by Clostridium perfringens Antisera

<table>
<thead>
<tr>
<th>Antisera types (0.15 ml.)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatants (0.5 ml.)</td>
<td>892 PM</td>
<td>2088 PM</td>
<td>892 PM</td>
<td>2088 PM</td>
<td>892 PM</td>
</tr>
<tr>
<td>Mice 1-5</td>
<td>0</td>
<td>0</td>
<td>0.16</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td>Mice 6-10</td>
<td>16</td>
<td>1.25</td>
<td>0.25</td>
<td>0.25</td>
<td>12</td>
</tr>
<tr>
<td>Mice 11-15</td>
<td>4</td>
<td>0.6</td>
<td>0.5</td>
<td>0.03</td>
<td>0.25</td>
</tr>
<tr>
<td>Mice 16-20</td>
<td>16</td>
<td>5</td>
<td>2.3</td>
<td>0.03</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Expressed in hours after injection.
EXPERIMENT 6

Effect of Pericardial Effusion Fluid in Pigs

An attempt was made to reproduce the lesions of Mulberry Heart Disease by the intravenous injection of pericardial effusion fluid from pigs which had died of the disease.

Materials and Methods

On separate occasions two pigs were each given pericardial effusion fluid from cases of Mulberry Heart Disease. The fluid, which was negative on culture was injected intravenously in the manner previously described.

Pig 4A, a six week old Tamworth cross pig weighing approximately 25-30 lbs. was injected with two ml. of pericardial effusion fluid. The animal was observed every 15 minutes for the first two hours and once daily for a period of one week.

Pig 142N, a 12 week old Yorkshire weighing approximately 90-95 lbs. previously used in Experiment 1 was given 10 ml. of pericardial effusion fluid. The animal was observed at the following times after injection: 15 minutes, 30 minutes, two hours, six hours, and then once daily for a week. The temperature of the animal was recorded at 15 minutes, 30 minutes, two hours, six
hours and on the second day after the administration of the pericardial effusion fluid.
Results

Pig 14A did not at any time exhibit any abnormal behavior after the administration of the pericardial effusion fluid.

The behavior of pig 142N was never noted to be abnormal, but its temperature 15 minutes, 30 minutes and two hours after injection was 104.4°F. Six hours after injection its temperature was 103.8°F., and on the next day the temperature was 101.8°F.
EXPERIMENT 7

Effect of Administration of Clostridial Cultures and Atropine to Pigs

An attempt was made to reproduce Mulberry Heart Disease by the oral administration of Clostridial cultures followed by a subcutaneous injection of atropine.

Materials and Methods

The Clostridium perfringens cultures used were isolated from the intestinal tract of cases of Mulberry Heart Disease on sheep blood agar plates incubated anaerobically. The organisms were then subcultured into Brewer's meat mash and incubated for 48 hours at 37°C. Smears made from the cultures before their administration to the pigs showed numerous organisms. The two strains of Clostridium perfringens used in this experiment proved to be Type A.

Two 17 week old Yorkshire pigs, 142N (previously used in Experiments 1 and 6) and 146N (previously used in Experiments 1 and 3), each weighing approximately 140-150 lbs. were each fed five lbs. of a commercial concentrate feed. Each pig was then given via a stomach tube five ml. of a culture of Clostridium perfringens. The cultures were washed through the stomach tube with 500 ml.

* Typed by Burroughs Wellcome Labs, Beckenham, Kent.
of cows milk. After dosing each pig was given one-tenth of a grain of atropine subcutaneously. The animals were observed at two and six hours after dosing and once daily for the next three days.
Results

The pigs appeared normal until 15 minutes after the injection of atropine at which time they began to sneeze. This continued for 20 minutes after which the sneezing stopped and the animals again appeared normal. On all subsequent observations there was never any abnormal behavior noted and the pigs continued to eat well.
EXPERIMENT 8

Acute Anaphylaxis in Pigs

Lamont et al. (22) suggested that Mulberry Heart Disease might involve some type of anaphylactic reaction. It was therefore decided to attempt to produce an acute anaphylactic reaction in pigs using horse serum.

Materials and Methods

Two 11 week old Yorkshire pigs, 141N and 144N (previously used in Experiment 1), weighing approximately 75-80 lbs., were each injected subcutaneously behind the left ear with eight ml. of horse serum. A two inch 18 gauge needle and a 10 ml. glass syringe were used. The pigs were held by a hind leg and the right ear during the injection procedure. Fifty days later the same pigs were each given 10 ml. of horse serum intravenously. The pigs were restrained with a hog snare and one animal (144N) injected via the ear vein and the other animal (141N) via the jugular vein. The pigs were observed for one and one-half hours and their actions recorded. They were observed periodically thereafter for two days.
Results

Both pigs showed hyperemia of the skin within five minutes after injection. In ten minutes, diffusely scattered one to two mm. red areas depressed below the surface of the skin appeared. These areas gradually turned blue in color and faded away completely within 30 minutes. Aside from some scratching with the hind legs and rubbing of the neck against the wall, no other abnormal signs were noted. The scratching and rubbing continued for about an hour. The next day the pigs were normal and had eaten well that morning.
DISCUSSION OF EXPERIMENTS

Although the foregoing group of experiments has not explained the etiology of Mulberry Heart Disease, some of the results are worthy of discussion.

Experiments 1 and 2 have shown that pigs are naturally highly resistant to Clostridium perfringens Type D toxin. It would seem unlikely that Mulberry Heart Disease is an intoxication due to the toxin of Clostridium perfringens Type D, when an animal can survive 100,000 to 200,000 mouse MLD doses as did pigs 146N and 149N in Experiment 1.

The results of Experiment 2 have shown that the pigs used in Experiment 1 did not have any detectable antibodies against the Type D toxin of Clostridium perfringens, as measured by serum neutralization tests, prior to, and 14 days after the injection of the toxin. It would appear then, that the resistance of pigs to the toxin is not dependent on the presence of circulating antibodies.

The slight lengthening of the survival time of some of the mice receiving the $6 \times 10^{-5}$ dilution of toxin and 0.25 ml. of serum, over that noted when toxin alone was given, is not considered to be significant. The dose of toxin injected was only one or close to one mouse MLD dose, to warrant recognition of the survival of the mice.
as an indication of a protective function of the serum.

Initial increases in body temperature, skin hyperemia and dyspnea exhibited by the pigs used in Experiment 3 which received the intestinal supernatant intravenously, are imputed to the shock produced by the injection of a foreign material into the blood stream and restraint of the animals. The increase in body temperature of pig 148N 18 hours after injection is ascribed to the presence of the infarcts in the kidney and toxemia. Partial paralysis and temporary blindness exhibited by pig 148N may be explained by acute edema of the central nervous system due to the action of toxic materials in the supernatant on capillary permeability. The lesions in the heart, kidneys, and brain stem of pig 148N are most likely the result of emboli from particulate matter in the supernatant. The other lesions in the brain and spinal cord of the two pigs (14ON and 148N) necropsied were slight and could not be related to a specific cause.

The results of Experiments 4 and 5 indicate that the supernatants used were rarely lethal to mice, and did not contain any toxins of Clostridium perfringens Types A, B, C, D, E. The significance of these findings with regard to toxin concentration in the intestinal tracts of the pigs just after death is doubtful. In sheep dying of Enterotoxemia due to Clostridium perfringens Type D toxin it is known that the concentration of toxin
in the intestinal tract falls off rapidly after the death of the animal (5).

Regarding the samples of supernatant used in Experiment 4, the exact time of death of the pigs from which they were prepared is not known, but was judged to be from eight to 24 hours.

If this experiment could be repeated using supernatants prepared from the small intestine of pigs which had been dead less than four hours, the results would be of greater significance as regards the amount of toxin present in the intestinal tract at the time of death.

The fact that the strains of *Clostridium perfringens* used in Experiment 7 were Type A deserves some comment. *Clostridium perfringens* Type A is a common post-mortem finding in the intestinal tract (26). Schofield (32) has described a condition of "Sudden Death in Calves Associated with Myocardial Degeneration". In a subsequent paper (33) he attributed the cause to the toxin of *Clostridium perfringens* Type A. Gangley et al. (11) working with fractions of a filtrate of *Clostridium perfringens* Type A and its relationship to the pathogenesis of Clostridial myonecrosis in man, found factors in the filtrate which inhibited phagocytosis and increased the sensitivity of the peripheral vessels to epinephelin. This sensitivity enhances the chance of circulatory collapse.
Since the heart lesions of Mulberry Heart Disease are so prominent, the administration of a potent Type A toxin to pigs as an experiment to produce necrosis of the myocardium would appear to be of value.

Experiment 7 itself may be criticized in that the atropine may have been given too soon after the administration of the culture, thus trapping the bacteria in the stomach where they were destroyed by the gastric acid. A pig with a duodenal fistula would overcome this difficulty.

Instead of bronchospasm and asphyxiation which is the result of experimental anaphylaxis in guinea pigs, the pig apparently reacts to the experimental production of anaphylactic shock as does the horse, where edema and urticaria of the skin are the prominent features (8).
SUMMARY

The history, necropsy findings, and bacteriological findings in 45 cases of Mulberry Heart Disease have been described and discussed.

The salient clinical feature of the condition is sudden death in pigs on an above-average plane of nutrition. Although Mulberry Heart Disease occurs in pigs of all ages, it is most often seen in the eight to twenty weeks of age group. Premonitory signs when observed include dullness, weakness in the hind legs, labored respirations, swollen eyelids, and cyanotic discoloration of the ventral surfaces of the body.

The post-mortem distinction is made on the heart lesions which include a markedly distended pericardial sac which contains a clear or straw-colored fluid and clots of fibrin, or fluid which gels on exposure to air. The heart shows streaks of hemorrhage under the epicardium, sometimes involving only the right ventricle, but both ventricles and both atria may be involved. There are ecchymotic hemorrhages beneath the endocardium which are usually found in the papillary muscles and interventricular septum. There are, in addition, pleural and peritoneal effusions, edema of the lungs, and congestion of the viscera.

Histological lesions include a hemorrhagic necrosis
and degeneration of the myocardium with vascular degeneration and thrombus formation, congestion and edema of the lungs, hemorrhage in lymph nodes, serous hepatitis and edema of the gallbladder, edema fluid under the glomerular endothelium, and lesions of the brain affecting the white matter predominantly. The brain lesions vary from vascular congestion to almost complete lysis of the white matter of the cerebrum. The variation in the severity of the brain lesions is thought to be dependent on the survival time of the animal. Bacteriological findings were not considered to be significant.

From the history, gross, and histological findings, it is suggested that Mulberry Heart Disease is a result of a primary toxic damage to vascular endothelium, with secondary effects due to hypoxia from pulmonary edema and heart failure.

Experimental attempts to reproduce Mulberry Heart Disease included the use of Clostridium perfringens Type D toxin intravenously; the administration of intestinal contents, intestinal supernatants, and pericardial fluid from cases of Mulberry Heart Disease; the oral administration of Clostridium perfringens cultures in conjunction with an injection of atropine, and the production of experimental anaphylactic shock in pigs.

The experimental procedures did not reproduce Mulberry Heart Disease, but seem to warrant the conclusion
that pigs are naturally resistant to *Clostridium perfringens* Type D toxin and that this resistance is not dependent upon the presence of circulating antibodies.


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Plate 1. Edema of the eyelids and exophthalmos in a pig with Mulberry Heart Disease.
Plate 2. Distended pericardial sac, edema and dorsal displacement of the lungs, and an enlarged, congested liver.
Plate 3. Pericardial sac opened showing coagulated pericardial fluid and hemorrhages on the heart. Note the edema of the lungs and gallbladder.
Plate 4. Pericardial sac opened showing coagulated pericardial fluid, hemorrhages on the heart, and edema of the lungs.
Plate 5. Diffuse hemorrhage under the epicardium, edema of the lungs.
Plate 6. Hemorrhage in the myocardium, most severe in the right ventricle and interventricular septum.
Plate 7. Section of formalin fixed brain showing areas of malacia and foci of hemorrhage in the white matter.
Plate 8. Same section as in Plate 7, but stained with Sudan Black B to show loss of myelin. Normal myelin is visible in the internal capsule for comparison. Black spots in the dorsal gyri are foci of hemorrhage.
Plate 9. Focal area of myocardial degeneration in a papillary muscle in the atrium. Note the peripheral rim of surviving tissue beneath the endocardium. H. & E. x120.

Plate 10. Swelling of the endothelium of a vessel in the atrium. H. & E. x500.
Plate 11. Swelling of the media of a vessel in the ventricular myocardium. P. A. S. x500.

Plate 12. Necrosis of a vessel in the auricular myocardium. This vessel is from the focus of degeneration depicted in Plate 9. H. & E. x350.
Plate 13. Hyaline thrombus in a vessel in the ventricular myocardium. H. & E. x350.


Plate 17. Empty sarcolemmal sheaths in the ventricular myocardium. P. A. S. x500.

Plate 18. Congestion and edema of the liver. H. & E. x500.
Plate 19. Central lobular necrosis of the liver. H. & E. x120.


Plate 22. Hemorrhage among the seminiferous tubules. H. & E. x85.

Plate 25. Edema of the white matter in the cerebrum. 
H. & E. x350.

Plate 26. Focal area of demyelination in the cerebrum. 
H. & E. x140.
Plate 27. Frontal cortex showing loss of myelin in the white matter of the gyri, but sparing the corpus medullare and associational fibers. Luxol Fast Blue. x3.
Plate 29. Fragmentation of axons and myelin sheaths with the formation of digestion chambers. Holmes' Luxol Fast Blue, x350.

Plate 30. Digestion chamber containing fragments of axon and myelin in an area of demyelination and necrosis in the cerebral white matter. Holmes' Luxol Fast Blue, x800.

Plate 33. Perivascular cuffing of a vessel in the cerebral gray matter. Predominant cell type in the cuff is the eosinophil. H. & E. x500.

Plate 34. Vacuolation of neurons in the spinal cord. Toluidine Blue. x120.
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