A STUDY OF AN ENCEPHALITIC STRAIN OF HOG CHOLERA VIRUS

By

HOWARD WALTER DUNNE

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Pathology

1951

In memory

of

my mother

ACKNOWLEDGMENTS

The author wishes to express his sincere thanks to Dr. Frank Thorp, Jr., whose untiring endeavor and high ideals have provided a guiding force throughout this investigation.

He is also greatly indebted to Dr. R. A. Runnells for his generous efforts in promoting the investigation and in examining the tissue sections, and to Dr. E. T. Hallman for his help and encouragement in early graduate studies.

Deep appreciation is expressed to Dr. H. J. Stafseth whose advice and support materially aided the progress of the experimentation.

The author is profoundly thankful to the late Dean C. S. Bryan and to Director C. M. Hardin for their encouragement and assistance in obtaining financial aid for the project.

Grateful acknowledgment is due to Dr. W. R. Hinshaw, Colonel R. Randall, V.C., U. S. Army, and Lt. Colonel Don L. Mace, V.C., U. S. Army, for their sound counsel and aid.

To Miss Esther Smith, infinite appreciation is expressed for assistance rendered throughout the investigation, particularly in the sectioning of tissues and in photography.

Further appreciation is due to Lt. S. C. Benbrook, U. S. Army Reserve, for his efficient laboratory aid.

Thanks also are extended to Dr. Walter Mack for advice and ultracentrifugation of virus samples and to Dr. Hans Ruhland and Mr. Irving Delappe for their aid in language translations.

Appreciation is also expressed for the assistance of Dr. Clifford Duncan in making calcium and phosphorus determinations of serum samples.

The author wishes to thank the United States Army for the financial assistance acquired through the V.M. Division, Biological Laboratories, Chemical Corps, at Camp Detrick, Frederick, Maryland.

To the American Veterinary Medical Association and the members of the Research Council the author wishes to express his sincere gratitude for the research fellowship which enabled him to prepare for this investigation.

Further appreciation is expressed to Michigan State College for the scholarship provided during the preparatory period of training.

The author is indebted to the Bureau of Animal Industry, United States Department of Agriculture, for the experimental serum used in testing for variant reactions of the virus, and to Dr. R. W. Luecke, Department of Agricultural Chemistry, Michigan State College, for the aureomycin used in the first experiment.

To Charles Kupsky, and student assistants John Norton, Doris Lyon, and Joe Nichols, the author extends his heartfelt thanks for their conscientious efforts to facilitate the research.

Too all of my other friends and coworkers who so generously contributed time and effort to this investigation I tender my deep appreciation.

Howard Walter Dunne

candidate for the degree of

Doctor of Philosophy

Final examination, October 30, 1951.

Dissertation: A Study of an Encephalitic Strain of Hog Cholera Virus

Outline of Studies

Major subject: Animal Pathology

Minor subjects: Biological and Physiological Chemistry, Physiology

Biographical Items

Born, March 19, 1913, Omaha, Nebraska

Undergraduate Studies, Iowa State College, 1937-41

Graduate Studies, Michigan State College, 1946-51

Experience: Private Veterinary Practice in the States of Washington and Iowa, 1941-42; Assistant Production Manager of Biological Division of the Corn States Serum Company, 1942-44; Production Manager, 1944-46; American Veterinary Medical Association Research Fellow, 1946-48; Assistant Professor of Animal Pathology, Michigan State College, 1948-50; Leave of Absence obtained to accept appointment with the United States Bureau of Animal Industry to investigate foot and mouth disease

in Europe and Mexico, 1948-49; Associate Professor in Bacteriology and Public Health, Michigan State College, 1950-51.

Member of Phi Zeta, Gamma Sigma Delta, Phi Kappa Phi, Society of the Sigma Xi, American Veterinary Medical Association, Michigan Veterinary Medical Association, Research Workers in Animal Diseases in North America, Michigan Society of Bacteriologists.

A STUDY OF AN ENCEPHALITIC STRAIN OF HOG CHOLERA VIRUS

By

Howard Walter Dunne

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Pathology

Year 1951

Approved Frank Thorp Jr.

HOWARD WALTER DUNNE

ABSTRACT

An atypical strain of hog cholera virus was isolated from a pig infected as the result of a field vaccination failure and passed through 16 serial intracranial swine passages without losing its atypical characteristics. This virus had the ability to cause a disease characterized by a short incubation period, encephalitic symptoms, frequent peracute deaths, and 100 percent mortality. Convulsions appeared in more than 40 percent of 100 subcutaneously and intracranially inoculated pigs. The passive immunity provided by a serum with known protective value for other hog cholera virus strains was insufficient to protect against the isolated field strain. The serum was used in established test doses.

Pathologically, the virus demonstrated an affinity for vascular endothelial tissue and was shown to cause gastric ulcers and button ulcers by infarction of the blood vessels of the gastric and intestinal mucosae. Brain sections indicated that perivascular cuffing of cranial blood vessels and the focal concentration of microglial cells were the two most frequently appearing lesions of the brain, and were most constantly found in the thalamus and in the medulla oblongata.

Frank K Thorp Jr

HOWARD WALTER DUNNE ABSTRACT

Attempts to enhance further the virulence of the virus by simultaneous inoculations with wheat germ oil or by elimination of the filtration process did not meet with great success.

Pseudomonas aeruginosa was isolated from five of 100 hog cholera infected pigs. No other pathogens were isolated. Inoculation of rabbits have eliminated the possibility of the presence of Aujeszky's disease. Examination of histological sections as well as clinical symptoms have eliminated Teschen's disease.

Frank Thayer Jr.

TABLE OF CONTENTS

Page

INTRODUCTION

The annual national production of 57 million swine valued at approximately two billion dollars (Agricultural Statistics 1949) emphasizes the importance of solving the problems associated with hog cholera, the disease which is still the number one killer of swine in the United States.

The first recorded cases of hog cholera appeared in this country in Ohio in 1833 (Moore, 1916) after which the disease spread slowly over the nation. Between 1846 and 1855 only 93 outbreaks were reported. It proved, however, to be a cyclic plague causing great outbreaks in 1887, 1896, 1913, and 1926 (Quin, 1950).

Atherton (1923-24) estimated the hog cholera loss in animals alone from 1914-24 to approximate 415 million dollars. Kinsley (1933) estimated the annual loss from hog cholera to be 20 million dollars. Quin (1950) approximated the annual loss at 30 to 40 million dollars. Deaths due to hog cholera have decreased from 120 per 1,000 swine in the great outbreak of 1887, and 130 per 1,000 in a similar outbreak in 1897 to 72 per 1,000 during 1913-16 (Haring, 1916). The death rate further decreased from 51 per 1,000 during 1917-20 to 39 per 1,000

during 1921-24, 23 per 1,000 during 1925-28, and 18 per 1,000 during 1929-31 (Kinsley, 1933). However, there still remains a problem of decreasing the losses which are frequently associated with serum and virus vaccination. Although the greatest numbers of swine lost were among nonvaccinated animals, there were perhaps as many as 25 percent (Atherton, 1923-24) lost as the direct result of vaccination following the use of serum and virus. Ray (1950) stated that after a decrease for five years, there was an increase in the prevalence of hog cholera in several states west of the Mississippi River. In states where there was an increase in hog cholera there was also an increase in postvaccination disasters.

The subject of postvaccination losses, frequently discussed at professional meetings and in the literature, became even more important with the increased losses from vaccinations during the years of 1949-50 (USDA, BAI, 1950). The factor involved in some of these losses has been described as a variant of hog cholera virus present in the virus preparation used in vaccination (Dale et al., 1950). Difficulty in maintaining this unstable variant in serial animal passage explained

the lack of protection given by sera which later demonstrated low potency against the variant.

In view of the above-mentioned factors, the investigation reported in this paper was instigated to study an atypical strain of hog cholera virus, to determine how this virus differs from the more typical strains and, how to maintain these atypical characteristics in serial passage.

REVIEW OF LITERATURE

Since the outstanding research of Dorset, Niles and McBryde (1908) many advances have been made in the production of anti hog cholera serum. Notable among the advances was the development of the bean extract precipitation of red blood cells in the production of clear serum (Dorset, 1916), and the elimination of the shock factor by decreasing the maximum heating temperature from 60° to 59° C. as the result of research by Munce and Hoffman (1930).

These improvements greatly reduced the hazards of using serum and virus but serum producers and practitioners continued to be plagued by an excessive number of post vaccination losses.

Various factors, most of which were important in the vaccination of swine against hog cholera, were stressed in explaining the untimely deaths of healthy pigs following the simultaneous injections of anti hog cholera serum and hog cholera virus.

Of these factors, bacterial infections in vaccinated animals had been the most commonly recognized causes of

vaccination failures. The work of Salmon and Smith (1885-1889) with the bacillus which later was given Salmon's name, stressed the frequency with which Salmonella choleraesuis was found associated with hog cholera infections. The pathogenicity of this organism for swine was indicated also, and its importance as the primary etiological agent in hog cholera was recognized until the work of De Schweinitz and Dorset (1903) proved a virus to be the causative factor.

Nevertheless, Salmonella choleraesuis continued to play an important part in explaining vaccination failures where the organism was frequently isolated from sick or dead animals. Its importance was emphasized by such men as Hoskins (1915), Dorset (1921), Cahill (1923), Van Es and Olney (1944), and Ray (1948).

Hutyra et al. (1946) regarded Bacterium suipestifer (Salmonella choleraesuis) and possibly other members of the paratyphoid group, such as Bacterium (Salmonella) enteritidis and Bacterium (Salmonella) murium as playing an important part in the pathogenesis of hog cholera by inducing a septicemia in hog cholera infected swine and superimposing bacterial infections upon the virus infection already present.

According to Dorset (1921) repeated trials showed that when susceptible pigs were injected simultaneously with the filterable virus of hog cholera and live cultures of Salmonella choleraesuis, a much more acute disease ensued with deaths in five to seven days. Virus alone generally takes fifteen days to cause death.

Van Es and Olney (1944) were able to produce a 70 percent death loss following serum and virus vaccination of animals fed Salmonella choleraesuis infected feed for periods of one to two months prior to vaccination. Cahill (1923), however, was unable to demonstrate either hog cholera virus or bacterial infections in a large proportion of postvaccination deaths and believed that the loss was due to some factor other than bacteria or hog cholera virus. Doyle (1920) found a large proportion of cholera-infected hogs having simultaneous gas producing bacteriemias. The bacteria proved to be Salmonella paratyphosa A and B and were highly fatal to rabbits. However, hog cholera blood containing gas formers was no more harmful than hog cholera blood without gas formers when injected in large quantities into hog cholera immune hogs.

For years Pasteurella multocida was a popular though unproved etiological factor in swine disease. Although its importance has dimmed, it has not faded completely as it was described by Hutyra et al. (1946) as an organism that gave rise to a rapid fatal septicemia in pigs already infected with hog cholera. They also described Escherichia coli, Corynebacterium pyogenes, and Bacterium pyocyaneum (Pseudomonas aeruginosa) as organisms found in the blood of swine infected with hog cholera, apparently existing as adventitious organisms having no etiological significance. Bacillus (Clostridium) oedematis maligni was credited by these authors as producing severe complications in swine fever by penetrating the stomach wall, and then being carried to the muscles where gas production took place.

Botulinus, type A, association with vaccination losses was discussed by Graham (1921), who found the organism present in 16 percent of commercial anti-hog-cholera products and described specific botulinus intoxication which he believed lowered the resistance of the animal and permitted the development of a latent low grade hog cholera.

Simultaneous infections of swine with influenza virus and hog cholera virus through the medium of anti hog cholera products has also been shown. Scott (1943) isolated swine influenza virus from pigs dying following vaccination against hog cholera when the simultaneous serum and virus method was used. He was of the opinion that hog cholera infection, resulting from vaccination, caused mobilization of influenza virus from the lungs into the blood stream resulting in the development of lesions of both diseases.

Young (1950) has suggested that swine might be susceptible to many strains of human influenza virus as well as to the swine strains, indicating a still wider scope of possible simultaneous infections.

Improper handling of animals during the vaccination procedure has also been condemned. Reynolds (1912), Ray (1948), and others have warned against the failure to vaccinate all animals in a drove, as well as the practice of unclean surgery, and the injection of serum into one of the systems of elimination such as the intestine or the urinary bladder in intraperitoneal vaccination.

Serum dosage has been recognized as perhaps the greatest single factor in controlling vaccination losses particularly in animals showing evidence of poor health. Insufficient serum dosage may result from syringe failure or underestimation of the animal's weight (Reynolds, 1912; Hoskins, 1915).

Serum dosage recommendations have shown a steady trend upward since 1912. The following chart shows that the minimum serum dosage recommended by the Federal Bureau of Animal Industry 1951, was increased 25 percent over their 1949 recommendations and approximately 100 percent over the recommendations of Reynolds in 1912.

	Reynolds (1912)	BAI 1949	BAI 1951
Suckling pigs	10 cc	16 cc	20 cc
Pigs 20 to 40 lbs.	15 cc	24 cc	30 cc
Pigs 40 to 90 lbs.	20 cc	28 cc	35 cc
Pigs 90 to 120 lbs.	25 cc	36 cc	45 cc
Hogs 120 to 150 lbs.	30 cc	44 cc	55 cc
Hogs 150 to 180 lbs.	35 cc	52 cc	65 cc
Hogs 180 lbs. and over	40-60 cc	60 cc	75 cc

The possibility of undesirable effects of large serum doses was explored by Van Es and Olney (1944) who demonstrated that 2 cc of hog cholera virus given with anti hog cholera serum doses of 0.43 cc per pound of body weight permitted the best gains in pigs. When 0.86 cc of serum per pound of body weight was given, animals remained in good health but did not show the gains observed in the first group. When 1.72 cc of serum per pound of body weight was used, two out of fifteen (13.33%) died of hog cholera in one experiment and three out of twenty (15%) died of hog cholera in a second experiment, indicating the inadvisability of using large doses of serum with this virus. It was interesting to note that when as little as 0.2 cc of serum per pound of body weight was given, only four out of fifteen died from hog cholera. One other animal died in this group from another cause, making the total loss five. A number of animals remained alive but unthrifty.

The stability of hog cholera virus in terms of virulence or as to antigenicity has been the subject of much speculation. Hupbauer (1933-34) tested strains of hog cholera virus from the United States, Argentina, and Spain against serum of German origin and concluded that plurality of types does not exist in

the sense that types exist in foot and mouth disease. The serum dose was generally 5 cc per 10 kilograms of body weight. When only 3 cc of serum for each 10 kilograms of body weight was used, the results varied from a mild reaction with no deaths to a 40 percent mortality with all strains used.

Kobe and Schmidt (1934) were able to produce a chronic type of hog cholera by dilution of the virus and concluded that chronic hog cholera was not the manifestation of a type of virus but rather a question of reduced virulence, and associated with the amount of the infecting dose of virus.

Kernkamp (1947), however, cited specific field cases of chronic hog cholera with 17 day contact incubation periods occurring in vaccinated as well as nonvaccinated droves.

A hog cholera virus of an entirely different antigenic nature was reported by Montgomery (1921) in British East Africa and by De Kock (1940) in South Africa. The disease produced by this virus followed the same pattern of symptoms and lesions as the European and American forms of the disease with the exception of the incubation period. African hog cholera had a short incubation period of 36 hours to four days with the average being about two days from the time of injection.

The vaccination disasters of 1949-50 resulted in investigations by Dale et al. (1951) who showed definite indications of a variant nature of the virus involved. Of foremost interest in their work was the failure of some commercial sera to protect against certain viruses isolated from field cases. Examples of these tests are as follows:

Weight (Pounds)	Amount of Serum (cc)	Kind of Serum	Death or Severe Reaction
60-80	30	Commercial	8/8
90-100	30-36	"	4/7
50-60	35	"	3/3
58	5	BAI Exp. 1	2/2
32	5	"	2/2
81	15	"	3/3
41	15	"	1/4
67	15	"	4/4
82	15	"	15/15
74	30	"	3/3
46	30	"	0/2
76	30	"	2/4
82	30	"	11/15
57	45	"	0/2
51	45	"	0/2
97	45	"	0/4

Inasmuch as the virus they tested produced no ill effects when used to challenge cholera-immune pigs, and since it produced typical cholera in cholera-susceptible pigs but failed to be neutralized by hog cholera antisera that protected against regular viruses, they concluded that they were working with a variant hog cholera virus.

No mention of nervous disorders was made in the aforementioned paper although apparently such has been common in a number of so-called vaccination "breaks." An editor's note (1951) stated that "outbreaks of unidentified nervous disorders in swine have been reported by practitioners in widely separated locations in the cornbelt." Quin (1950) concurred that sporadic cholera convulsions occur in peracute cases, and stated that the most typical thing about hog cholera is that it frequently is atypical.

The development of convulsions in pigs affected with hog cholera is not a recent observation. Brunschwiler (1925) observed that an animal may run in circles first to the right and then to the left, subsequently throwing itself down and going into a "cramp." It would draw itself backward with an opening and closing of the mouth. Dilated pupils, and pushing movements with the front legs extended were also observed.

Seifried (1931) reported the experimental production of 39 cases of encephalitis in swine characterized by histological changes in the brain. Nervous symptoms of varying degrees were seen. He did not make it clear, however, whether these nervous symptoms were severe enough to be called convulsions. Also it was not certain whether he had maintained such activity through serial animal passages.

Hutyra et al. (1946) stated that

> Exceptionally there occur convulsions and various compulsive movements with marked lethargy. Attacks of delirium also occurred concurrent with hemorrhages between the meninges or into the brain substance. (Usually, however, histologically demonstrable encephalomyelitis is symptomless.)

Encephalitic symptoms were reported with other diseases of swine involving a number of animals in a drove. Ray (1945) observed exaggerated nervous symptoms in swine infected with streptococci. In such outbreaks, a number of swine have developed brain involvement. These animals walked continuously, sometimes in circles or aimlessly about in the pen. They sometimes stepped high with the forefeet which resulted in a peculiar distressed motion. Such cases showed no diagnostic lesions but resembled other acute septicemic diseases of swine.

Hofferd (1944) stated that convulsions may be exhibited in cholera infections, but are seen in a number of other diseases including dietary deficiencies. It is possible that the author was referring here to calcium deficiency tetany.

Kaplan (1948) described encephalitic clinical reactions of swine infected with "porcine encephalomyelitis" or Teschen's disease. Such animals showed incubation periods of three to thirty-one days. Lassitude, depression, and mild ataxia developed into nervous excitement. Paralysis followed muscular tremors, convulsions, salivation, gnashing of teeth, chewing of bedding, and circling. The animals would fall on their sides, squeal, go into convulsions, recover, and then regain their feet. The paralytic stage came gradually, the animals losing control of their hind legs exhibited a "dog sitting" posture and a dragging of the rear quarters. Approximately 50 percent of affected animals died during the first two weeks. Early recoveries in nonparalyzed animals occurred in one to two weeks. Some animals remained paralyzed for weeks.

Findlay (1938) classified variants according to environmental conditions which brought about their changes. The conditions cited involved the passage of viruses through (a) different

tissues in a different species, such as yellow fever in the brains of mice; (b) different tissues of the same species, developing neurotropic strains by brain passage, e.g., yellow fever in rhesus monkeys; (c) the same tissue, but different species, e.g., vaccinia produced from variola in calf skin; (d) embryonic tissue in vitro, e.g., pantropic yellow fever in Tyrode-serum-chicken or mouse embryo tissue; (e) chorioallantoic membrane of developing chicken embryo, e.g., decrease of influenza pathogenicity by propagation in these tissues; (f) mouse carcinoma, e.g., pantropic yellow fever lost virulence for rhesus monkeys as the result of prolonged passage; (g) alteration of physical conditions, e.g., vaccinia propagated at 37.5° C.; and (h) treatment of one virus by another killed virus, e.g., active fibroma virus and heat-inactivated myxoma virus produced myxomatosis.

Serial passage of the fibroma virus in domestic rabbits by Andrews (1936) resulted in the production of an acute inflammatory reaction in place of the fibroma-like growth.

The existence of a common stem virus for St. Louis encephalitis and Western equine encephalomyelitis viruses was suggested by Hammon (1948) who isolated an agent from chick mites. This agent was modified by passages in different species

until it developed into distinct types, namely, St. Louis encephalitis virus in mice, and Western equine encephalomyelitis in chicken embryo. He considered that coincidental "mixed infections" with St. Louis encephalitis and equine encephalomyelitis, occurred too frequently for mere coincidence.

Taylor (1949) observed an antigenic change from the parent stock of an influenza virus strain as the result of passage through embryonated eggs in the presence of immune serum. It was thus conceivable that the natural passage of influenza virus through human subjects where antibodies are encountered as the result of previous infections might give rise to strain deviation.

Intracerebral inoculations of infectious agents has received much attention in the last score of years. In demonstrating the pathogenicity of B.C.G. vaccine, Neiman and Woolpert (1936) injected guinea pig fetuses intracerebrally and were able to demonstrate multiplication of tuberculosis organisms.

In an attempt to find a laboratory animal suitable for yellow fever research, Theiler (1930) injected mice intracerebrally and found that these rodents were susceptible to inoculation by this route while by all other routes of inoculation they

were resistant to infection. The strain was carried for more than seventy brain-to-brain passages. This established virus was highly neurotropic for these animals but manifested a loss of virulence for monkeys.

In discussing tissue affinities of viruses, Galloway (1936) stated that yellow fever, louping-ill, equine encephalomyelitis and African horse-sickness viruses were pantropic in the sense that they possessed multiple cellular affinities. This was most true with yellow fever, whereas with louping-ill these affinities were more limited to the reticulo-endothelial system. Nevertheless, he considered all of them as possessing neurotropic potentialities.

Lammert and Moussatche (1943) were able to adapt tissue culture-grown yellow fever virus in one- to four-day old chicks by serial brain-to-brain passages. The virus was not encephalitic in the chick but was severely encephalitic when injected into monkeys.

Reichel (1913) compared the increase of virulence of hog cholera virus in serial passages to fixed rabies virus which was passed through serial animal passage until a maximum virulence was obtained.

Wright and Habel (1948) demonstrated that substrains of Pasteur fixed rabies virus were neither identical in their antigenicity, their ability to overcome rabies immunity in immunized mice, nor in the incubation periods, duration of symptoms or lesions of the type of disease they produced.

Other than body temperature, the determination of total white blood cells per cu. mm. of blood, referred to hereafter as T.W.B.C., has remained about the most satisfactory laboratory aid available for following the course of infection in hog cholera inoculated swine.

The T.W.B.C.'s of 40 normal swine were found by King and Wilson (1910) to average 19,982 with a range from 10,070 to 39,296. In cholera-infected swine the average T.W.B.C. was 15,515 in 20 animals with a range of 7,200 to 23,600. No information was given on methods of inoculation, amounts of virus, day after inoculation that the sample was taken, or duration of illness.

Dinwiddie (1914) cited normal T.W.B.C.'s ranging from 7,800 (suckling pig) to 17,600. He observed that T.W.B.C.'s decreased from 8,500 on the first day of illness to a low of 2,000 on the sixth day of illness. Of the pigs studied, the

range on the sixth day of illness was from 2,000 to 15,300. The latter animal lived for more than 32 days.

Cahill (1928-29) reported normal T.W.B.C. at 13,600 with a range of 10,300 to 16,600. He observed a drop to 4,400 in 24 hours using commercial virus. At no time did the count exceed 5,100. Just prior to death T.W.B.C. dropped to 600 and 2,200 in two animals. The total number of animals inoculated or the inoculation dose was not mentioned.

Thorp and Graham (1930) found an average T.W.B.C. of 27 cases diagnosed as hog cholera to be 7,700.

Hewitt (1932) found a wide variation in T.W.B.C.'s of swine injected with viruses from different sources. T.W.B.C. of hog cholera-infected pigs from a commercial serum company averaged 4,609. Hewitt reported that samples from the United States Bureau of Animal Industry Experiment Station at Ames, Iowa, averaged 17,489 T.W.B.C., whereas blood samples from cholera infected pigs of the Iowa State College Veterinary Research Institute averaged 17,178. Blood samples were obtained from the sixth to the twelfth days after virus inoculation. The average normal T.W.B.C.'s of 51 swine was 21,524, whereas the average of 53 hog cholera-infected pigs was 10,422.

A comprehensive study of the T.W.B.C. of cholera infected swine was made by Lewis and Shope (1929). Using a commercial virus, intraperitoneal passages were made with blood drawn on the fifth day after inoculation. In earlier passages, disease was regularly induced after four to seven days incubation. Later, after several passages, the incubation period was regularly three days. The infective virus titer was greater than 10^{-5} but less than 10^{-6}. The latter dilution was not infective. Little difference was shown between the effect on T.W.B.C. from doses of 0.001 cc of virus and 4.5 cc of virus. In the latter case a low of 2,840 was reached on the seventh day. The smaller virus dose resulted in T.W.B.C. as low as 4,250 also reached on the seventh day. A marked decrease in T.W.B.C. from normals averaging 16,505 to 8,550 and 11,880 was observed on the second day. The course of the disease apparently ranged from 10 to 37 days since T.W.B.C. determinations were terminated on those days.

Cole (1932) found that feeding of pigs caused a marked increase in the T.W.B.C. of 25 pigs from an average of 17,742 before feeding in the morning to 22,893 one and one-half hours later after feeding. In vaccination tests pigs weighing 65 to 80

pounds were treated with 20 cc of serum and 2 cc of virus. All animals remained well but the T.W.B.C.'s dropped from a normal of 16,171 to 13,106 on the second day and 8,320 on the third day to a low of 7,206 on the fifth day. Normals were reached again on the tenth day.

The average T.W.B.C. of 15 pigs receiving an experimental strain of virus alone showed a relatively slow decrease in numbers with a drop from 19,229 to 12,225 on the second day with comparatively little change until a low of 5,862 was reached on the eighth day. Kernkamp (1939) injected 55 swine intramuscularly with hog cholera virus and found a marked drop from an average normal of 14,200 to 10,700 on the first day to 7,400 on the second day, and a low of 5,280 on the sixth day.

Dunne (1948) found that pigs artificially infected with hog cholera and suffering complications with pneumonia often did not develop a low T.W.B.C. until the sixth day after inoculation with the hog cholera virus.

The search for laboratory aids in the diagnosis of hog cholera prompted investigations into the use of the complement fixation test by such investigators as Healy et al. (1915) and Connoway and Durant (1915). Both groups believed that they

had developed a satisfactory system. Their success, however, was not confirmed by others.

Cell inclusion bodies observed in cells of the conjunctiva by Himmelberger (1916) and in the endothelial cells of gall-bladder by Boynton et al. (1941) and Sippel (1945) provided hope for a field diagnostic aid.

Growth of hog cholera virus on tissue culture was reported by Hecke (1932) and Boynton et al. (1948), and on chorio-allantoic membranes of chicken embryos by Tenbroeck (1941). Although important contributions to the experimental field these systems offered little immediate aid in testing for the presence of virus in an unknown sample.

Attempts to use laboratory animals for hog cholera diagnostic purposes by Jacatot (1937) and Muir (1943) met with no success. Zichis (1939), however, was able to pass the hog cholera virus through ten serial passages in sheep while Baker (1946) and Koprowski (1946) were able to demonstrate serial passage of the virus in rabbits. Clinical symptoms were so slight, however, that they provided no aid from a diagnostic standpoint.

The intradermal skin test of Sarnowiec (1934) suggested a possible field test for hog cholera but does not eliminate the use of swine as the test animal.

Kernkamp and Roepke (1942) demonstrated in vitro neutralization of hog cholera serum as a laboratory aid with limited possibilities.

The use of brain sections as a diagnostic aid has been studied by such men as Brunschwiler (1925), Seifried (1931), Röhrer (1934), and Köbe (1934) with somewhat conflicting results. Helmbolt (1950), however, demonstrated that perivascular cuffing and microglial foci resulting from hog cholera infections were found primarily in the brain stem and in more than 90 percent of the cases.

MATERIALS

The virus used in this experiment was isolated from a pig following field vaccination failure. The following description of this case and the subsequent treatment of the virus prior to its usage in intracranial passage offers an interesting background for this experiment. The pigs averaged about 75 pounds in weight, and received 48 cc of serum and 3 cc of virus. A 50 percent loss was suffered in this herd. Severe pneumonia was evident in many of the sick pigs. Diarrhea was not prevalent. Some of the animals, including the one presented for autopsy, had been injected with penicillin. No history of convulsive action was given. The animals were on a high protein, alfalfa meal, corn and oats ration. This pig was staggering with weaving motion of its hind quarters, had a T.W.B.C. of 8,000 and a temperature of 106.0 degrees F.

Lesions, upon necropsy of the animal submitted to the Michigan State College Animal Pathology Laboratory, included mild focal pneumonia, peripheral hemorrhage of the lymph nodes, petechial hemorrhages of the kidney, bladder and gall-bladder. The kidneys also showed some infarction. There was severe

early button ulcer formation in the colon and cecum, and moderate ulceration of the stomach.

A section of the intestine showing severe ulceration was ground with sterile quartz and filtered through a Seitz E-K filter.

One passage through swine was made with this virus before intracranial passage was attempted. Six pigs were inoculated with the intestinal filtrate in the following manner: Two were injected intranasally, two intradermally and two subcutaneously. One animal from each of the respective groups of two was placed on a feed containing 10 grams of purified aureomycin per 100 pounds of feed. A vaccinated control was placed with the group not receiving the aureomycin.

In summarizing the results of daily temperatures and total white blood cell determinations (Table I), the aureomycin treated group developed an earlier temperature rise, and a generally lower T.W.B.C., but ran a somewhat more chronic course than did the non-aureomycin group. In the aureomycin treated animals one pig showed convulsions on the 11th day after inoculation of the virus.

Post mortem lesions in these pigs were not markedly different from those of animals from which the seed virus was obtained. However, neither the aureomycin group nor the control group developed enteritis.

Hog cholera-vaccinated pigs were obtained from the college herd where they had been routinely vaccinated with serum and virus by college veterinarians.

Susceptible pigs used in this experiment were obtained from farmers in rural areas near the laboratory. Since vaccination against hog cholera is not practiced extensively in central Michigan, it was relatively easy to find susceptible animals for experimentation with the virus of hog cholera. When possible, groups of 30 or more were purchased at one time in an attempt to control individual variations in resistance. Size and age varied somewhat, although eight week-old weanling pigs were desired. During the winter months, 12- to 14-week old pigs were the youngest animals available. During the spring months, however, eight-week old weanling pigs were obtained. Since a two-week quarantine period was observed, and since not all the pigs were utilized in experiments at once, some of the animals grew to 75 pounds or more in the earlier groups before they

were used. The problem of handling these larger pigs made them undesirable from an experimental standpoint. Little attention was given to the breed of pigs purchased. With few exceptions all were crossbred pigs. The exception was one group of O.I.C. Chester Whites. Since most of the crossbred pigs came from more or less remote Hampshire lineage, it was inevitable that many should display a black hair coat with white belting.

METHODS

Virus Preparation

The brains from infected animals were maintained in a deep freeze at -20° C. until the day before inoculations were to be made. Where possible, the brain selected for the experimental serial passage was one that had been removed from the first animal to die in the last completed passage of the series. The first animal to die was usually one of those which had suffered convulsions. Thus selection was made on the basis of early death and convulsions.

The brain was removed from the deep freeze and allowed to thaw at 42° C. A portion, never more than half of the brain, was weighed accurately and ground in a mortar with sterile quartz sand. Brain-heart infusion broth was added in sufficient quantity to make a 20 percent suspension of the tissue. Saline was used as a vehicle for a short period but the use of broth was resumed after three passages. This suspension was centrifuged at 3,000 to 4,000 r.p.m. for periods of 20 to 30 minutes in an International refrigerated centrifuge and filtered through a

Seitz E-K filter pad. The higher speed and longer period facilitated filtration.

Since it was believed that excessive quantities of virus adhered to the filter pad during the filtration process, an attempt was made to eliminate filtration by the use of antibiotics. Thus, to control bacterial contamination, 5,000 units of penicillin was added to 100 cc of the brain in broth suspension. This method was used in only two passages.

Bacteriologic tests of the finished product were made by inoculating 0.5 cc of the material into a tube of nutrient broth and incubating at 37° C. for 48 hours.

Animal inoculations included the injection of rabbits through intravenous, subcutaneous and intracranial routes with amounts varying from 0.3 cc to 3.0 cc. Chicken embryos were inoculated in the yolk sac, chorioallantoic membrane and cerebrum.

Ultracentrifuged virus material was prepared by subjecting 30 cc of filtered brain suspension, as described above, to centrifugation at 42,000 r.p.m., averaging 144,500 g., in a Spinco ultracentrifuge. The resulting pellet was resuspended in 1.0 cc of the supernatant fluid and injected subcutaneously

into a cholera susceptible pig. The virus used in this experiment was obtained from an animal inoculated subcutaneously with phenolized commercial hog cholera virus.

Swine Inoculations

Quarantine pens were maintained approximately 1,000 yards from the infected premises. Susceptible animals were maintained in these pens under strict quarantine until they were needed for experimental purposes. The caretaker was allowed to have no contact with the hog cholera infected pigs, the infected premises, or the laboratory in which the virus was handled.

The isolation pens, in which the inoculations were executed, were of two types, both contained in a single cement block structure. The large pens had outside windows for ventilation and were provided with an entrance vestibule. Two solid doors of the entrance vestibule separated the infected pen from the central hallway. In this entrance vestibule was a sink, soap, scrub brush, paper toweling, thermometers, coveralls, boots, all virus free, and an eight inch deep foot bath containing five percent sodium hydroxide for boot disinfection. A sterilized

feed can with feed, and a dispensing bucket were also maintained within the vestibule. Sufficient feed was provided to last through the experiment. Any surplus, not fed to the infected pigs, was given to recovered animals. These pens were sufficiently large to accommodate conveniently six to eight small pigs.

The smaller pens were arranged to provide space for one or possibly two small pigs. A cage was constructed at table height in each pen with a four foot wide sliding wire mesh door which could be raised to permit access to the animal. Construction of the room was similar to that of the previously described larger pen, including the vestibule and sink, with the exception that ventilation of all but one pen was obtained through the central hall. This last feature necessarily limited the usage of these units. Hoes were maintained in each unit for scraping fecal material into a disposal can. The refuse container remained in the pen until the termination of the experiment. Operating gowns were provided in each small unit to eliminate a change of coveralls before entering each pen.

Cleansing and disinfecting the contaminated pens provided difficulties which were expected. Following the termination of an experiment, the walls and floor of the pen with its residue,

bedding in some cases, feed, and troughs were thoroughly soaked with five percent sodium hydroxide solution and allowed to stand for a period of 24 hours. The debris was then removed and the pen thoroughly scrubbed and hosed. When the surplus water had drained, walls, ceiling and floor were thoroughly soaked with a five percent solution of sodium hydroxide using a three-gallon hand pumped spray unit. The spraying process was repeated two additional times over a 48-hour period and the pen then allowed to remain idle for a week. Buckets, rubber hose, shovels and troughs were subjected not only to the sodium hydroxide spray, but also to a steam treatment for four hours.

Controls placed in these pens during the course of the experiments gave evidence that the desired elimination of all the hog cholera virus had not been accomplished.

Since there was a pressing need for the use of these pens, they could not be allowed to remain unoccupied for a sufficient length of time to allow the residual virus to be destroyed. It appeared that one week was too short a period to accomplish this desired end. To circumvent this obstacle, the procedure was concluded by allowing the room to dry after the spraying and then painting the walls and floors with "Textone,"

a water soluble alkaline resistant plastic type paint. Wooden doors were treated with linseed oil and metal parts were painted with aluminum paint. This procedure proved to be satisfactory.

Susceptible swine were transported from quarantine pens to the experimental barn by means of a clean trailer used only for this purpose. Virus free workers wearing sterilized coveralls and sodium hydroxide cleansed boots removed the animals from the trailer. In the central hallway which was wet with five percent sodium hydroxide, the pigs' temperatures were taken and then the animals were bled via the anterior vena cava with four-inch 18 gauge needles and five cc syringes. Vials containing 0.5 cc of a solution of ammonium and potassium oxalate (4.80 grams and 3.20 grams, respectively, per liter) were dried and used for the blood containers. This was adequate for five cc of blood. All instruments were sterilized before use. Numbers were clipped on the backs of the animals and a complete description of sex and markings noted before placing the subject into the experimental pen. Sometimes the animals were inoculated within a matter of a few minutes following their distribution in the pens. Under no circumstances, however, was inoculation delayed more than 24 hours.

Intracranial inoculations were made with the animal under anesthesia. Surital sodium (Parke Davis) in a 1:25 aqueous solution was injected either intravenously via the anterior vena cava or intraperitoneally. The former method insured quick complete anesthesia using approximately one cc of the solution to five pounds of animal weight. The latter method provided a quick, easy method of injection, and a number of animals could be injected in succession. With this method, approximately ten minutes was allowed for the animal to lose consciousness. No further handling was then necessary and inoculations could be made in a quick, efficient and humane manner. Duration of surgical anesthesia in either case was from ten to fifteen minutes, occasionally longer. No toxicity was observed and no animals died following the use of the anesthetic.

The hair on the pig's forehead was clipped closely with an electric clipper, using surgical blades, or with a sharp pair of scissors. The area was scrubbed with alcohol and painted with iodine. An incision was made on the medial line slightly above the point where an imaginary line drawn from the right eye to the base of the left ear crosses a similar line drawn from the left eye to the base of the right ear. The skin of

the forehead was then pulled to one side bringing the incision approximately one-half an inch lateral to the medial line. At this point the skull was trephined using a one mm. dental burr in an electric (Handi grinder) drill, or a handle equipped with a one-quarter inch chuck and a sharp pointed wedge shaped pin fashioned from a stainless steel intramedullary bone pin. This provided a 5/16 inch, sharp pointed probe approximately 1/16 of an inch wide at the base and with shoulders to prevent deeper penetration. When the cranium was pierced, a one-half inch needle was inserted into the cerebrum and the virus injected. The amount was 0.5 cc in the first five passages and 1.0 cc thereafter until the fourteenth passage when the 0.5 cc dose was resumed. After injection of the virus the needle was removed and the skin slid over the cranial opening.

Subcutaneously inoculated swine had 1.0 cc of virus injected into the left axillary space. Serum, when given, was injected into the right axillary space. Vitamin E, when given to decrease the animal's resistance, was injected in the form of wheat germ oil into the right axillary space. However, the wheat germ oil was not given to animals receiving serum.

In most of the pens constant running water was provided for drinking purposes. Feeding was done once daily. The ration consisted of:

Ground corn	60 parts
Ground oats	13 parts
Meat scraps	10 parts
Soybean oil meal	14 parts
Mineral	3 parts
Irradiated yeast (Vitamin D2)	120,000 units per 100 pounds

Daily observations were made each morning. Coveralls provided in each pen were donned, boots scrubbed in lye solution and hands scrubbed thoroughly with soap and water before entering the pen. Temperatures were taken, actions observed, and blood samples taken. The latter was accomplished by bleeding either from the ear or the tail. The area was cleaned with alcohol, dried with a piece of gauze, and a small incision made with a scalpel. The sample was collected with a white blood cell pipette and diluted with N/10 hydrochloric acid containing a small amount of gentian violet. The total white blood cell counts were made later in the laboratory.

When convulsions occurred, attempts were made to secure Kodachrome motion pictures, or black and white still pictures of the reacting animals.

Necropsy was performed upon all dead or moribund individuals in the experiment. The most desired subject was one that was in a moribund state, but frequently animals died shortly after attaining this condition. Those animals found "in extremis" were destroyed by electrocution, using 110 volts with terminals clamped on the ear and on the tail. Both sites were first dampened. Tissues were routinely saved from all animals dissected.

Histological Methods

Organs from which tissues were taken for sectioning included cerebrum, cerebellum, brain stem, colon, rib sections from costochrondral junction, auricle of the heart, kidney, liver, gall-bladder, urinary bladder, tonsil, stomach, spleen and lymph node.

All tissues, except brain and bone, were fixed in Bouin's fixative (Lillie, 1949). The brain was fixed in 10 percent formol-saline and the bone sections in Petrunkevitch (1933)

solution. The sections were washed in two changes of 70 percent alcohol for 24 hours. They were dehydrated and infiltrated according to the butyl alcohol-paraffin mush method of Johnson et al. (1943).

Microscopic sections were cut at 7u and stained with hematoxylin-eosin. Some sections were stained with Masson's (Goldner, 1938) trichrome stain.

Bacteriological Examination

A bacteriological check on all swine necropsied was made by culturing the heart blood, the brain and the large intestine. A loopful of brain tissue was cultured upon blood agar slants. Three to five cc of heart blood drawn aseptically with a sterile syringe was inoculated into a tube of beef heart broth. A section of intestine, preferably a portion displaying a lesion of enteritis, was dissected and placed into a tube of tetrathionate broth base medium. Following 24-hour incubation, a transfer of a loopful of this material was made to SS agar and to MacConkey agar. Picked colonies were inoculated into Kligler's iron medium and those cultures showing even remote possibilities

of being salmonella were further checked for fermentation reactions in sugar tubes. Motility media were also used.

No attempt was made to check the bacterial flora of lungs showing evidence of pneumonia or to isolate bacteria from stomachs showing ulceration.

RESULTS

Encephalitic Symptoms

The primary symptomatic deviation of the disease caused by this virus from that of hog cholera, as commonly observed in swine, was an increased manifestation of nervous symptoms between the third and eleventh day following the inoculation of the virus. Usually these symptoms were noticed first on the fifth or sixth day, starting as convulsive seizures of one to two minutes in duration. The interval between seizures varied from a few minutes to two or more hours. In some of the more severely affected animals, the intervals became shorter and shorter until almost continuous convulsive activity was evident. Usually at this time the animal was quite exhausted and its activity was more feeble. The convulsions commonly followed a pattern. The animal stiffened, the head was thrown back, the eyes closed tightly, and the legs extended rigidly. The subject remained in this position, exhibiting a muscular tremor for a period of ten to fifteen seconds (Plate I, Figures 1 and 2). This was followed immediately by an easing of

PLATE I

Figure 1. The initial stage of a convulsion with the pig throwing itself backwards.

Figure 2. A few moments later the animal came to a stop against the wall but still in a state of convulsion. Most animals do not retain an upright position at this stage.

PLATE I

Figure 1

Figure 2

muscular tension, permitting a burst of extended running action while the animal remained on its side (Plate II, Figures 1 and 2). Loud squealing sometimes accompanied the running movements which lasted for a period of perhaps thirty to forty seconds. With the cessation of the convulsive action, the animal relaxed slowly but breathed heavily and displayed a wild look in its eyes (Plate III, Figure 1). If quite exhausted upon cessation of convulsions the animal would frequently lie on its sternum with a depressed attitude (Plate III, Figure 2).

Upon becoming prostrate, the pig would lie on one side only. Any attempt to place it on the opposite side was met with violent efforts to regain the original position. The animal would struggle in a furious manner. Usually the original position would be attained unless the animal was too weak. The animal would then lie quietly until the next convulsion.

The seizures did not always precede an early death. Many times there was complete recovery from nervous symptoms. The animal even recovered so far as to be able to get up on its feet again, to take nourishment, and to live for several days. One animal survived for more than a month following convulsive seizures.

PLATE II

Figure 1. The two prostrate pigs are in the late stages of a seizure. The pig in the far background went into convulsions a moment later.

Figure 2. The running movements of this pig were so rapid as to blur the picture. Note the arc in the sawdust by the extended forefeet.

PLATE II

Figure 1

Figure 2

PLATE III

Figure 1. In the last stages of seizure the animal had slower movements and a wild look in its eye.

Figure 2. Following several seizures an animal with sufficient strength assumes an upright position and appears exhausted. Usually animals remain on their side after several convulsions.

PLATE III

Figure 1

Figure 2

More commonly, death occurred within two or three days after the onset of nervous symptoms. The animal did not always continue to show these symptoms until the time of death.

Tables I and II show that the occurrence of convulsions was not related to the route of inoculation. Of 52 intracranially inoculated animals, 22 individuals or 42.3 percent displayed severe nervous symptoms whereas 11 of 21 subcutaneous inoculated animals or 52.2 percent reacted in a similar manner. There seemed to be a somewhat cyclic variation in the incidence of convulsive activity throughout the series of passages, with the pigs of some passages reacting 100 percent and others showing no encephalitic symptoms.

A small number (less than ten percent) of the animals listed in Table I as having nervous symptoms did not exhibit bonafide convulsions. These showed chorea-like movements of the head or legs, or winced spasmodically as if in severe cerebral pain. Since, in a few cases, the cessation of convulsive activity had been observed to occur within a period of eight to ten hours from their onset, it may be that these few cases were passing through the end of a convulsion period. Shivering and

TABLE I

THE RELATION OF NERVOUS REACTIONS TO THE COURSE OF THE DISEASE IN SERIAL INTRACRANIAL SWINE PASSAGES

Passage No.	No. with Severe Nervous Symptoms over No. Injected	Day Nervous Symptoms Observed	Day of First Death	Average Days of Survival
1	2/3	6, 8	8	15.7
2	0/2	-	4	9.5
3	2/3	6, 7	6	25.0*
4	3/3	4, 5, 6	6	7.7
5	0/1	-	8	8.0
6	2/2	6, 8	6	15.5
7	0/3	-	21	26.7
8	2/2	9, 10	10	12.0
9	3/3	3, 5, 6	4	8.3
10	0/2	-	9	10.0
11	0/4	-	8	9.0
12	2/6	3, 5	4	11.3
13	2/6	8, 11	9	12.2
14	0/6	-	10	12.0
15	3/4	7, 7, 9	7	10.5
16	1/2	4	5	7.0

* One animal showing no convulsions survived 48 days.

TABLE II

THE RELATION OF NERVOUS REACTIONS TO THE COURSE OF THE DISEASE IN SUBCUTANEOUS INOCULATED SWINE

Virus of Intracranial Passage No.	No. with Severe Nervous Symptoms over No. Injected	Day Nervous Symptoms Observed	Day of First Death	Average Days of Survival
3	3/3	6, 8, 8	9	10.3
4	1/3	5	-	10.7
5	0/1	-	14	14
6	1/2	8	6	7
8	2/2	8, 9	8	9
9	2/3	5, 6	7	8.7
10	2/7	7, 13	13	13.6

nervous movements were common in most dying animals but were not included in the table. Observations were not usually made more than twice a day due to the distance between the laboratory and the quarantine quarters. It may be that with closer observation the number of convulsion cases reported would have been greater.

One animal suffered convulsions on the seventh day following inoculation with virus "A" and developed a posterior paresis on the tenth day. It was able to drag itself around with its forelegs, but could provide only feeble movements with its hind legs for the remaining two weeks of its survival period.

In 16 serial intracranial swine passages of the virus there was a tendency toward decreased encephalitic symptoms in passages 10 through 14 as shown in Table I. However, pigs in passages 15 and 16 again showed marked convulsive activity. The over-all occurrence of nervous symptoms dropped from 63.6 percent at passage 9 to 42.3 percent of all animals inoculated intracerebrally up to and including passage 15.

It is interesting to note in Table I that convulsions were associated with early deaths. Although many of the early deaths, as well as later deaths were the result of electrocution, all

animals destroyed were in a moribund condition, and did not appear strong enough to survive 24 hours. The average day of the first death in groups showing convulsions occurred 6.3 days following inoculation. The average day of the first death in groups not exhibiting convulsions occurred ten days postinoculation. Thus there appeared to be a correlation between the acute course of the disease and the occurrence of convulsions.

Convulsions occurred from the third to the thirteenth day after inoculation but averaged 6.7 days. It was observed that 80 percent of all convulsions occurred between the fifth and the ninth day after injection of the virus. Death of pigs suffering convulsions occurred on the average of 9.6 days following inoculation.

Goose-stepping was noticed in several animals. Possibility of pantothenic acid deficiency existed only if the prolonged period of anorexia was sufficient to deplete any reserve the animal might have had. The diet provided adequate B vitamin-containing constituents for healthy pigs.

Perversion of appetites, in the form of eating straw in preference to palatable feed, was also observed. Animals in a weakened condition would sometimes gnaw on the troughs, the

walls and even the attendant's boots. Yet, if placed where good feed or drinking water was available, the pigs would often ignore both. At such times the pigs would appear to be almost blind in the stumbling movements.

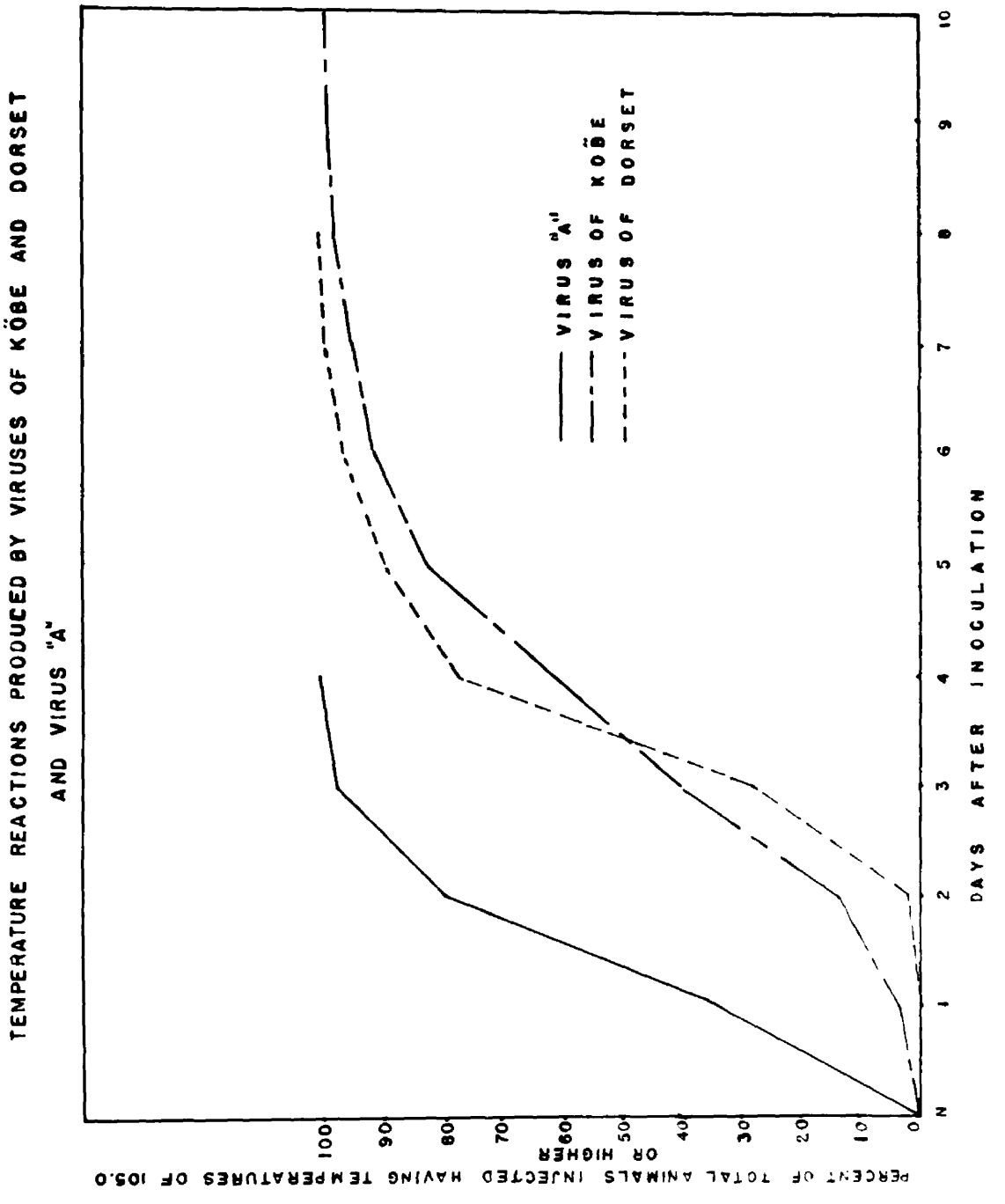
Diarrhea, following constipation, was common in many animals from the eighth day until death. The feces were usually yellow in color but frequently were black from partially digested blood. In a few cases unaltered blood was defecated.

During the colder months, when heat was provided in the pens, the mucous exudate dried on the nostrils of the pigs, sick seven days or longer. The exudate plugged the nostrils so that frequently the animals had great difficulty in breathing. This did not occur so frequently in later experiments.

Temperature Reactions

The early body temperature rise in swine following inoculation with Virus "A" denoted a short incubation period and suggested high virulence. In later passages the temperature reactions on the first and second days appeared more frequently than in earlier passages. Plate IV shows the temperature reactions caused by virus "A" as compared to reactions observed by

PLATE IV


TEMPERATURE REACTIONS PRODUCED BY VIRUSES OF KÖBE AND DORSET AND VIRUS "A"
PERCENT OF TOTAL ANIMALS INJECTED HAVING TEMPERATURES OF 105.0 OR HIGHER
0
10
20
30
40
50
60
70
80
90
100
N
1
2
3
4
5
6
7
8
9
10
DAYS AFTER INOCULATION
VIRUS "A"
VIRUS OF KÖBE
VIRUS OF DORSET

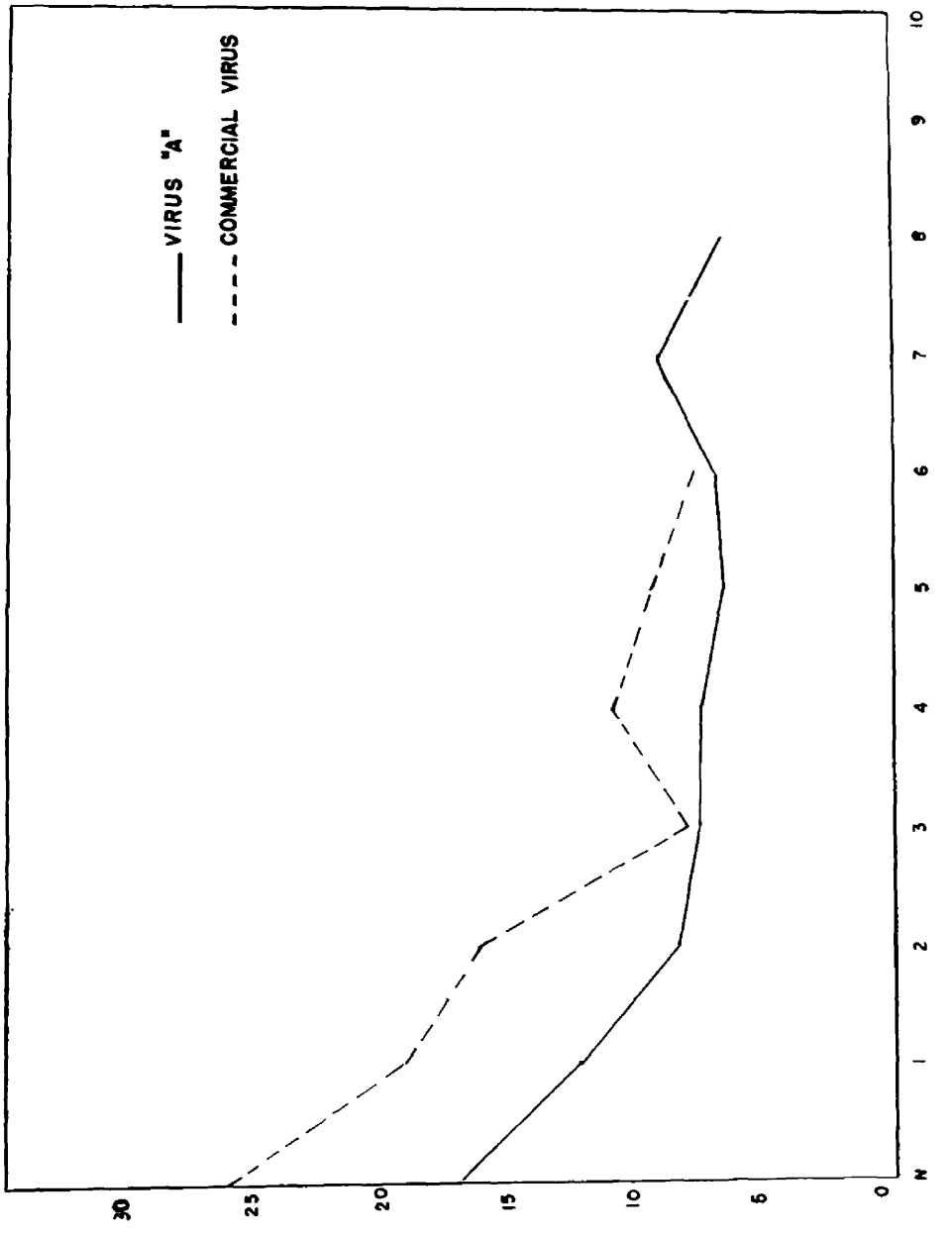
Dorset (1922) and Kobe (1934). Only those animals upon which daily temperatures were taken were included in the virus "A" curve. Plate IV demonstrates that on the first day following inoculation with virus "A," 35.5 percent of 39 animals showed a temperature of 105.0° F. or higher. Reactions in 79.5 percent and 97.4 percent of the animals inoculated with virus "A" appeared the second and third respective days. This is compared to Kobe's findings of 4.5 percent, 14 percent and 40.5 percent temperature reactions on the first, second and third days respectively after inoculation. Dorset's observations of 0 percent, 2.25 percent and 28.0 percent temperature reactions on the three days following inoculation are also compared. Commercial hog cholera virus I obtained locally and inoculated into one pig caused a rise of temperature over 105.0° F. on the fourth day.

Effect of Virus on T.W.B.C.

The total white blood cell counts also indicated a short incubation period. A curve constructed from the median of daily T.W.B.C. on 53 animals (Plate V) shows a rapid drop on the first and second days following inoculation with virus

PLATE V

THE EFFECT OF VIRUS "A" AND COMMERCIAL VIRUS II ON T.W.B.C.



"A." This curve is compared on the same chart with curves based on the daily T.W.B.C. of animals injected with commercial virus II. The curve of the virus "A" animals shows evidence of a much more acute reaction than that of the commercial virus. In 15 serial intracranial passages there was no indication of a decrease in the leucopenic response to virus "A." Commercial virus II is represented by the average T.W.B.C. of six animals.

Tables XIII through XXII give the detailed daily T.W.B.C. of intracranially inoculated swine through 13 of the 15 serial passages as well as the T.W.B.C. of subcutaneously inoculated swine and of animals in some of the immunity experiments. It will be noted that throughout the experiments the short incubation period of virus "A" is reflected in the rapid drop of T.W.B.C. When compared to commercial virus I (Table XXV) and commercial virus II (Table III and XXV) it is easily noted that virus "A" effects a much more rapid T.W.B.C. drop than either of the two commercial viruses.

In various experimental phases reported in this paper the T.W.B.C. is used as a means of following the course of the virus infection. In conjunction with the use of body

TABLE III

A T.W.B.C. COMPARISON OF VIRUS "A" WITH COMMERCIAL VIRUS ALONE AND IN THE PRESENCE OF SERUM

Day	Virus "A" (53 pigs)	Commercial Virus II (6 pigs)	Commercial Serum 15 cc Commercial Virus II 2 cc (10 pigs)	Commercial Serum 15 cc Virus "A" 2 cc (6 pigs)	BAI Serum 15 cc Virus "A" 2 cc (7 pigs)
N	17,167	26,100	25,100	15,200	16,607
1	12,700	19,487	23,094	-	-
2	8,650	16,287	21,240	9,525	15,128
3	7,890	8,275	19,820	8,725	12,728
4	7,830	11,094	17,600	7,765	10,400
5	6,750	9,745	20,550	10,125	8,971
6	7,184	7,860	23,100	14,150	9,457
7	9,264	-	23,280	11,450	11,733
8	6,968	-	26,850	11,450	15,100
	100 percent mortality	Pigs bled for virus production	No deaths	1 pig died	4 pigs died

temperatures, the T.W.B.C. is an effective laboratory aid for studying hog cholera virus.

Immunity Reactions

The effectiveness of Bureau of Animal Industry Experimental serum No. 1 and of a commercial serum was tested in a series of immunity experiments. Table II summarizes the results of experiments with BAI serum and demonstrates its relative ineffectiveness in neutralizing virus "A." In light weight pigs (35-40 lbs.), all animals became sick when injected subcutaneously with 2.0 cc of the virus and intramuscularly with 15 cc of the BAI serum. Four out of seven or 57.1 percent of these died. One of three 35 to 40 pound animals sickened but did not die when 30 cc of serum was used to neutralize the virus.

The commercial serum was much more effective but not completely effective when 15 cc amounts were injected into 60 pound swine as shown in Table III. Although this dose of serum is small, it is the Federal BAI (1949) regulation test dose for this size pig in commercial serum production.

Two routes of virus inoculation, the intracerebral and subcutaneous routes, were used in the immunity experiments. Of the two, the intracerebral route required the least serum for neutralization. This was at least partially due to the smaller intracerebral virus dose which was 0.5 cc to 1.0 cc, while the subcutaneous virus injections were made in 2.0 cc amounts.

Exposure of Vaccinated Pigs

Four vaccinated pigs were exposed to the "A" virus. Pig No. 4 had been vaccinated approximately two months prior to the experiment with commercial serum and virus and was in good health. As a contact control, the pig had a normal T.W.B.C. of 19,550 (Table XXIII). On the eighth day following the animal's contact exposure to the original field case "A" virus, the T.W.B.C. dropped to 12,250 and temperature rose from 102.4° to 104.0° F. Both T.W.B.C. and temperature returned to a more normal range for three days. Then the T.W.B.C. dropped again to 11,600 on the twelfth day, 8,500 on the thirteenth day and rose to 9,600 on the fourteenth day. The temperature again rose to 104.0° on the fourteenth day. Thereafter, the T.W.B.C. and temperature returned to normal,

but the animal developed a yellowish diarrhea, lost its appetite, had a severe exudative conjunctivitis and lost much weight. Recovery was uneventful after approximately ten days of illness.

Three additional pigs, numbers 107, 108, and 109 (Table XXIII), vaccinated with commercial serum and virus approximately two months prior to this experiment, were injected subcutaneously with 0.5 cc of "A" virus (20 percent suspension of brain in broth). The reaction was a marked drop in T.W.B.C. in two of the three pigs from 24,100 to 10,400 on the fourth day in one pig, and from 20,350 to 8,950 on the third day in the other. Both pigs developed a diarrhea on the eighth day following inoculation but subsequently enjoyed an uneventful recovery. All three pigs showed body temperatures of 104.4° to 104.8° following the virus injection.

Exposure of a Recovered Pig

Pig No. 4, completely recovered from his exposure to virus "A" two months previously, was injected with 3 cc of the 20 percent suspension of virus "A" infected brain in broth. A slight drop in T.W.B.C. was noted, from 19,850 to 16,400

on the third day with a return to 18,750 on the fourth day and 20,000 on the fifth day. No significant variation was noted thereafter. The body temperature of this animal, however, did rise to 104.0° F. following the virus injection.

Enhancement of Virus Propagation

Three methods were used in an attempt to increase the virulence of a filtered, 20 percent brain in broth suspension of hog cholera virus. These methods included ultracentrifugation, simultaneous injections with vitamin E, and the use of penicillin in place of filtration for obtaining a bacteria-free virus. For ultracentrifugation, a 30 cc sample each of virus "A" and commercial virus I was concentrated by means of a Spinco ultracentrifuge turning at 42,000 r.p.m. for four hours. The resulting pellets were resuspended in brain heart infusion broth, pH 7.4 and each injected into a susceptible pig.

The ultracentrifuged commercial virus I caused T.W.B.C. and temperature reactions similar to those established for virus "A." At 48 hours the T.W.B.C. had dropped below 6,000 and the animal's temperature had risen to 105.0° F. Death occurred on the tenth day.

TABLE IV

IMMUNITY EXPERIMENTS USING BUREAU OF ANIMAL INDUSTRY EXPERIMENTAL SERUM

Weight of Pigs	Route of Inocu-lation	Amount of Serum	No. Sick No. Injected	No. Died No. Injected
35-40 lbs.	IC	10 cc	3/3	1/3
35-40 lbs.	S	15 cc	7/7	4/7
35-40 lbs.	S	30 cc	1/3	0/3

TABLE V

IMMUNITY EXPERIMENTS USING COMMERCIAL SERUM

Weight of Pigs	Route of Inocu-lation	Amount of Serum	No. Sick No. Injected	No. Died No. Injected
80 lb.	IC	5 cc	2/2	2/2
60 lb.	S	15 cc	2/2	1/2
60 lb.	IC	15 cc	1/2	0/2
35 lb.	S	15 cc	3/4	0/4

Virus Dose was 2.0 cc in Subcutaneously Inoculated Animals and 0.5 cc to 1.0 cc in Intracranially Inoculated Animals.

IC - Intracranial

S - Subcutaneous

Virus "A" reacted very erratically following ultracentrifugation. On the second day the animal's temperature had risen to 104.8° but the T.W.B.C. remained normal. From the third day on the T.W.B.C. pursued an undulating course from 6,500 to 52,000 and down to 2,600. This animal lived for 22 days. Further work will be done to complete this portion of the study. The results seemed to indicate that the virulence of the commercial virus could be enhanced by ultracentrifugation, to react in a manner somewhat similar to virus "A." This might indicate that the pattern pursued by virus "A" is one of virulence variation rather than antigenic variation.

The use of vitamin E as a virulence enhancing agent was based on work done by Young (1950) which indicated that mice fed high levels of vitamin E in conjunction with vitamins A and D were more susceptible to influenza infections. Following this suggestion, wheat germ oil was injected intramuscularly in 5 cc amounts into pigs at the time of virus inoculation. Six animals (two serial passages) injected with the wheat germ oil showed a tendency toward shorter incubation periods than animals not injected.

Reasoning that much virus was probably lost during the filtration process, it was thought that a higher virus titer might be obtained by using penicillin to make a bacterial free virus. Penicillin was added to the virus suspension at the rate of 50,000 units per 100 cc of suspension. Six animals (two serial passages) injected with penicillin treated virus reacted in the same manner as animals injected with filtered virus. No additional benefit was believed to be achieved by the antibiotic treatment in lieu of filtration.

Aureomycin fed to pigs at the rate of 10 gm per 100 pounds of feed for two days prior to virus "A" inoculation and for as long as they would eat following inoculation failed to have any effect upon the course of the disease (Table XII) unless perhaps it aided the virus by shortening the incubation period. At least it can be said that the virus suffered no deleterious effects from the use of aureomycin in the feed.

Gross and Microscopic Pathology

It can be stated quite definitely that the type of hog cholera caused by virus "A" was essentially a disease of endothelial tissues, characterized by hydropic degeneration,

endothelial proliferation, perivascular cuffing and in many areas hemorrhage. With few exceptions, the lesions and symptoms observed in hog cholera infections apparently resulted from degenerating or proliferating endothelial tissues.

Virus "A" infection was characterized by the severity of lesions produced in the animals, particularly the severe hemorrhages of the kidney and lymph nodes, numerous infarcts of the spleen, extensive ulceration of the stomach and large intestine, and pronounced congestion of the brain. Also occurring in virus "A" infected animals was severe acute rickets, not so noticeable grossly but quite evident histologically. It was obvious that the rachitic condition had been developing only a matter of days, and was most evident in animals living two to three weeks following inoculation.

Brain Pathology

The most characteristic gross change in the brain was congestion which was present in 72 percent of the cases necropsied as shown in Table VIII. Animals destroyed during or shortly following a convulsive seizure, invariably showed severe congestion of the brain.

Microscopically, sections of medulla, thalamus and cerebrum were examined for lesions. The most significant changes were perivascular cuffing with small lymphocytes, and endothelial proliferation with subsequent partial or complete occlusion of the vessels (Plates VI and VII). Foci of microglia were observed in scattered areas in a few sections (Plate VII). No neuronophagia was observed in the sections studied.

In Table VI, the severity of brain lesions, as indicated by both the number of vessels involved and the amount of cellular infiltration, was compared with the occurrence of convulsions. Sections from 20 animals were studied. Ten of these had demonstrated convulsions and ten had not.

The thalamus showed lesions in 100 percent of the cases, while one out of 20 medulla sections did not show evidence of hog cholera infection. The cerebrum was the least affected by the virus with sections from two of twenty animals being negative for lesions and 12 showing only mild cerebral changes.

Table VI shows that the most significant reactions occurred in the medulla and thalamus. Although there seems to be little correlation between convulsions and severity of lesions, it is well to consider that not all animals suffering from

PLATE VI

Medulla oblongata, pig 221. Virus "A" infected. Perivascular cuffing with partial occlusion of the vessel lumen. Hematoxylin eosin X 800.

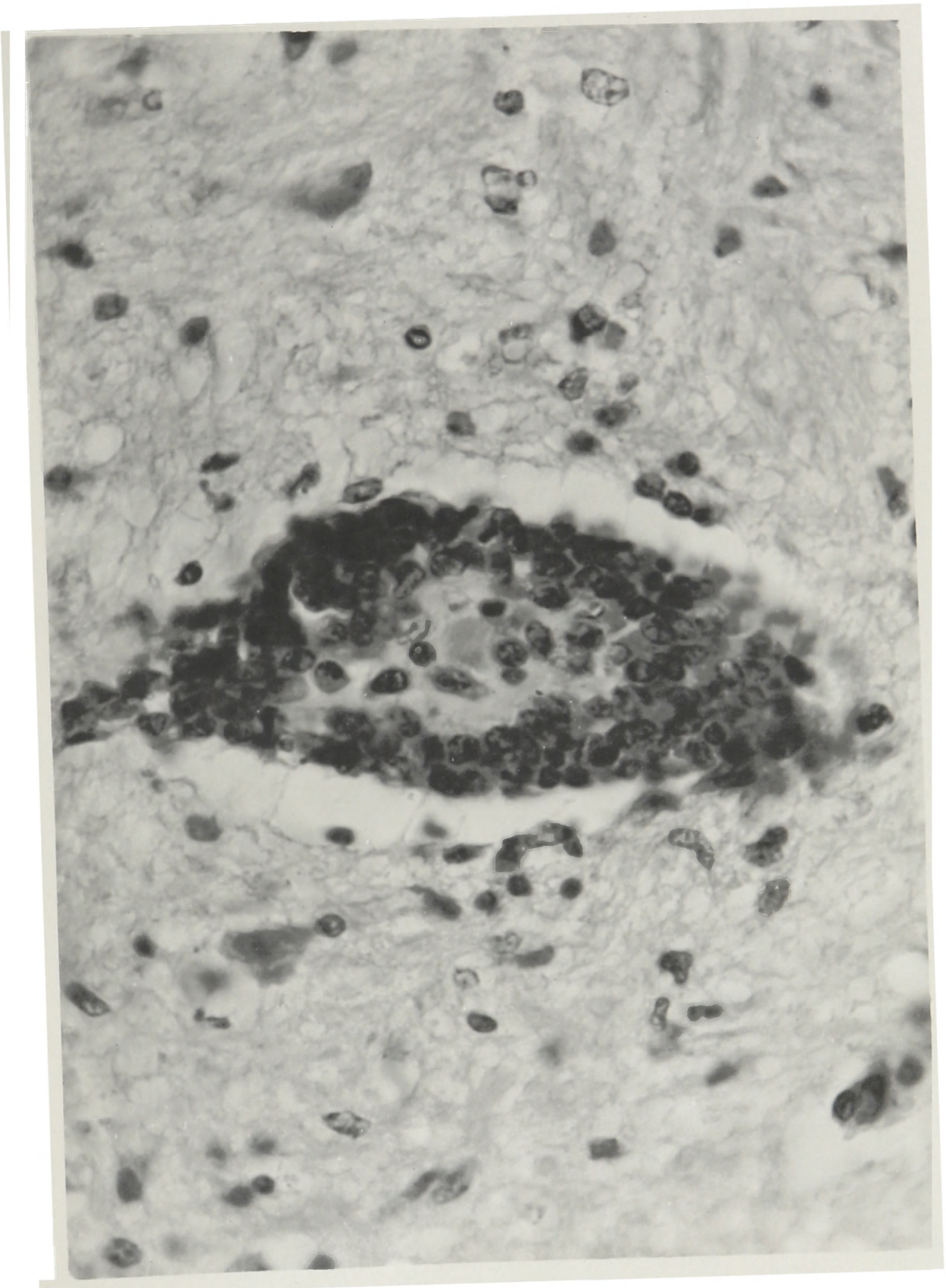


PLATE VII

Thalamus, pig 202. Virus "A" infected. A, glia cell concentrations; B, perivascular cuffing with partial occlusion of the vessels. Hematoxylin eosin X 160.

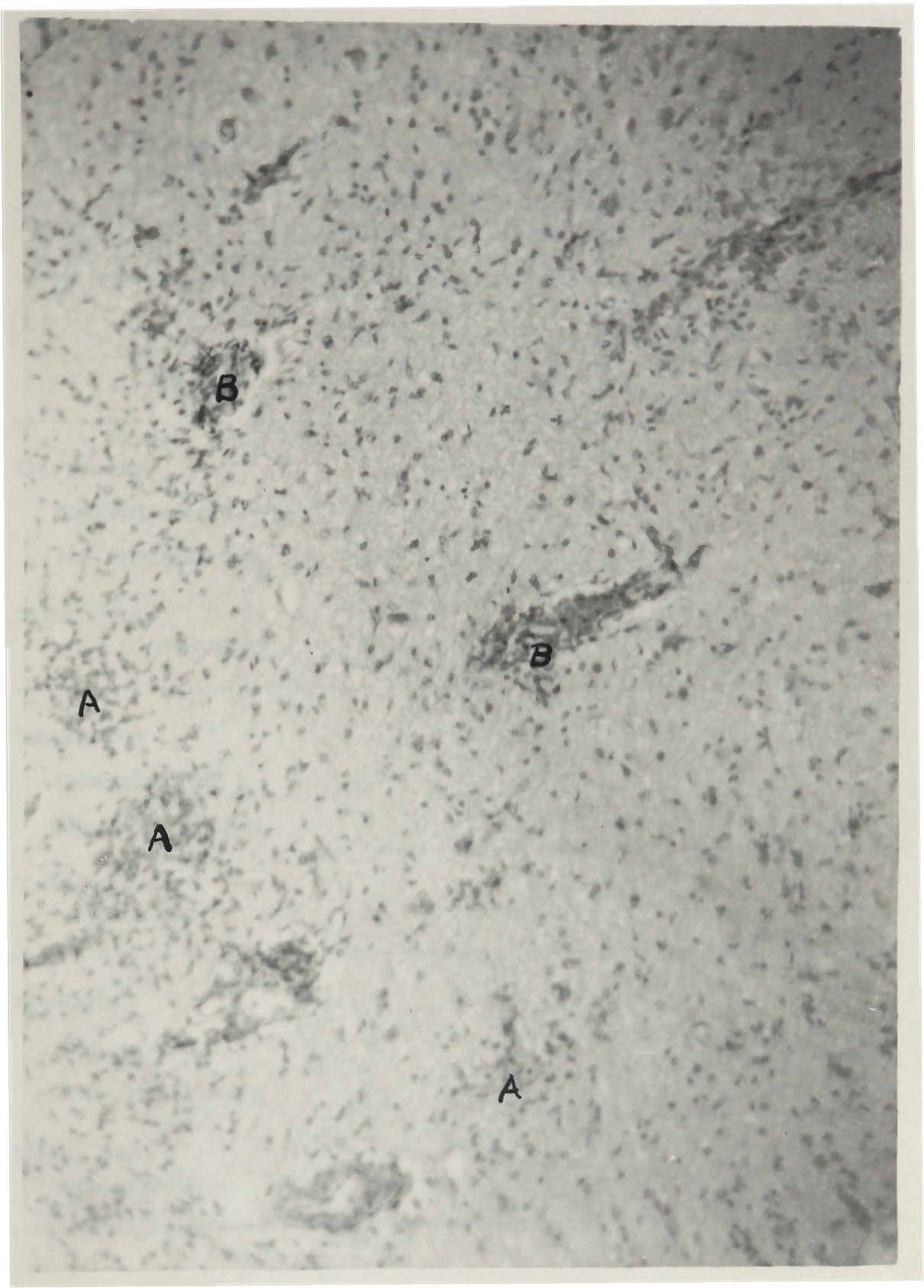

B
B
A
A
A

PLATE VII

Thalamus, pig 202. Virus "A" infected. A, glia cell concentrations; B, perivascular cuffing with partial occlusion of the vessels. Hematoxylin eosin X 160.

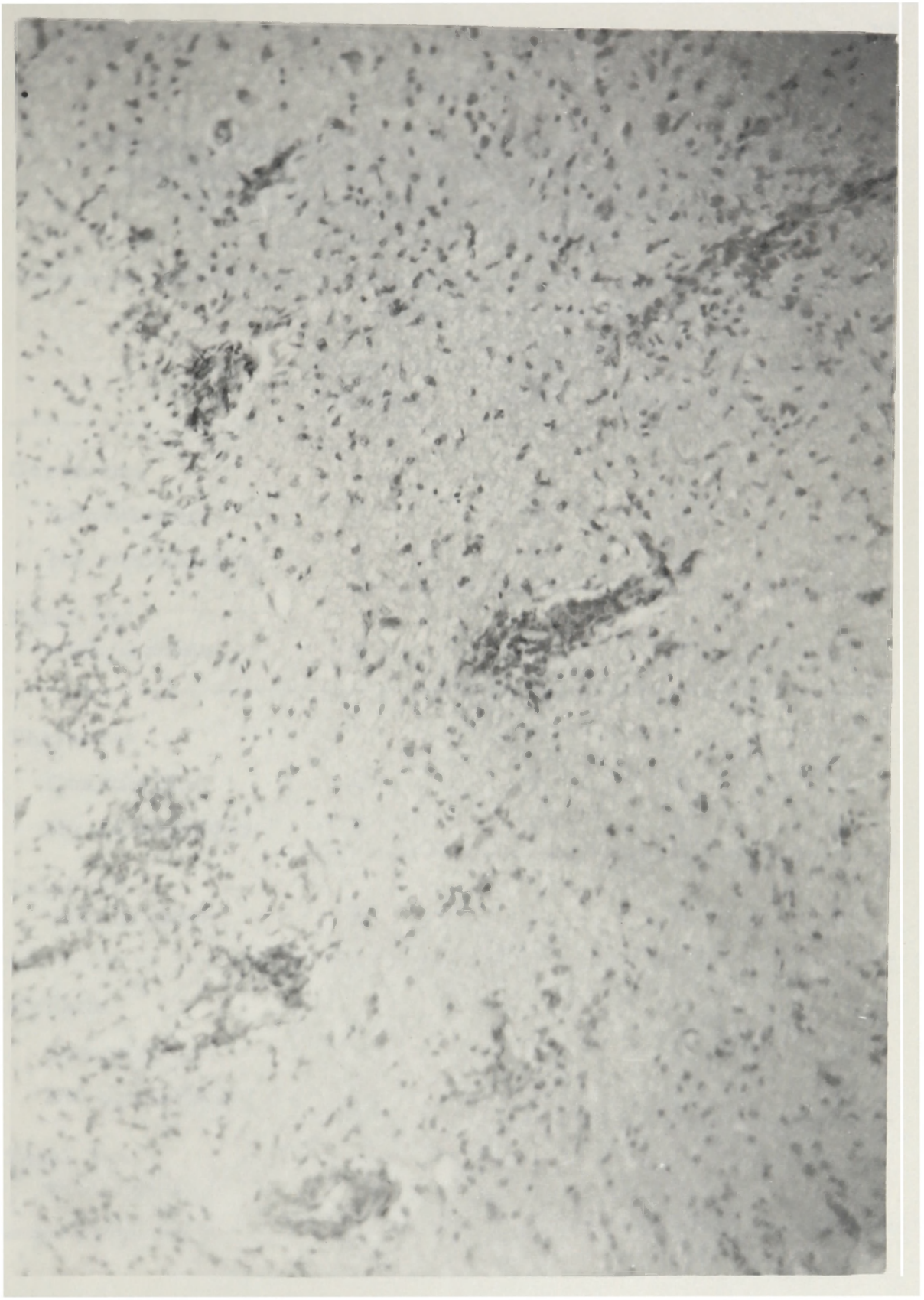


TABLE VI

COMPARISON BETWEEN SEVERITY OF LESIONS, OCCURRENCE OF CONVULSIONS AND AREAS OF PATHOLOGY IN THE BRAINS OF 20 ANIMALS AFFECTED

	Severity of Lesions in Numbers of Animals Affected			
	Negative	Mild	Moderate	Severe
Cerebrum				
Convulsions	0	5	3	2
No convulsions	2	7	1	0
Total No. with lesions	2	12	4	2
Thalamus				
Convulsions	0	3	4	3
No convulsions	0	1	4	5
Total No. with lesions	0	4	8	8
Medulla				
Convulsions	0	3	2	4
No convulsions	1	3	3	4
Total No. with lesions	1	6	5	8

convulsions died or were killed during the period of seizures. Table VII indicates that severity of lesions in brain tissue may be greatest during a relatively short time between the tenth and fourteenth days. Sections taken before or after this period generally show less severe lesions. Likewise only three sections of the brain were studied. A more complete examination of the parts of the brain might have revealed specific areas responsible for the encephalitic reactions.

TABLE VII

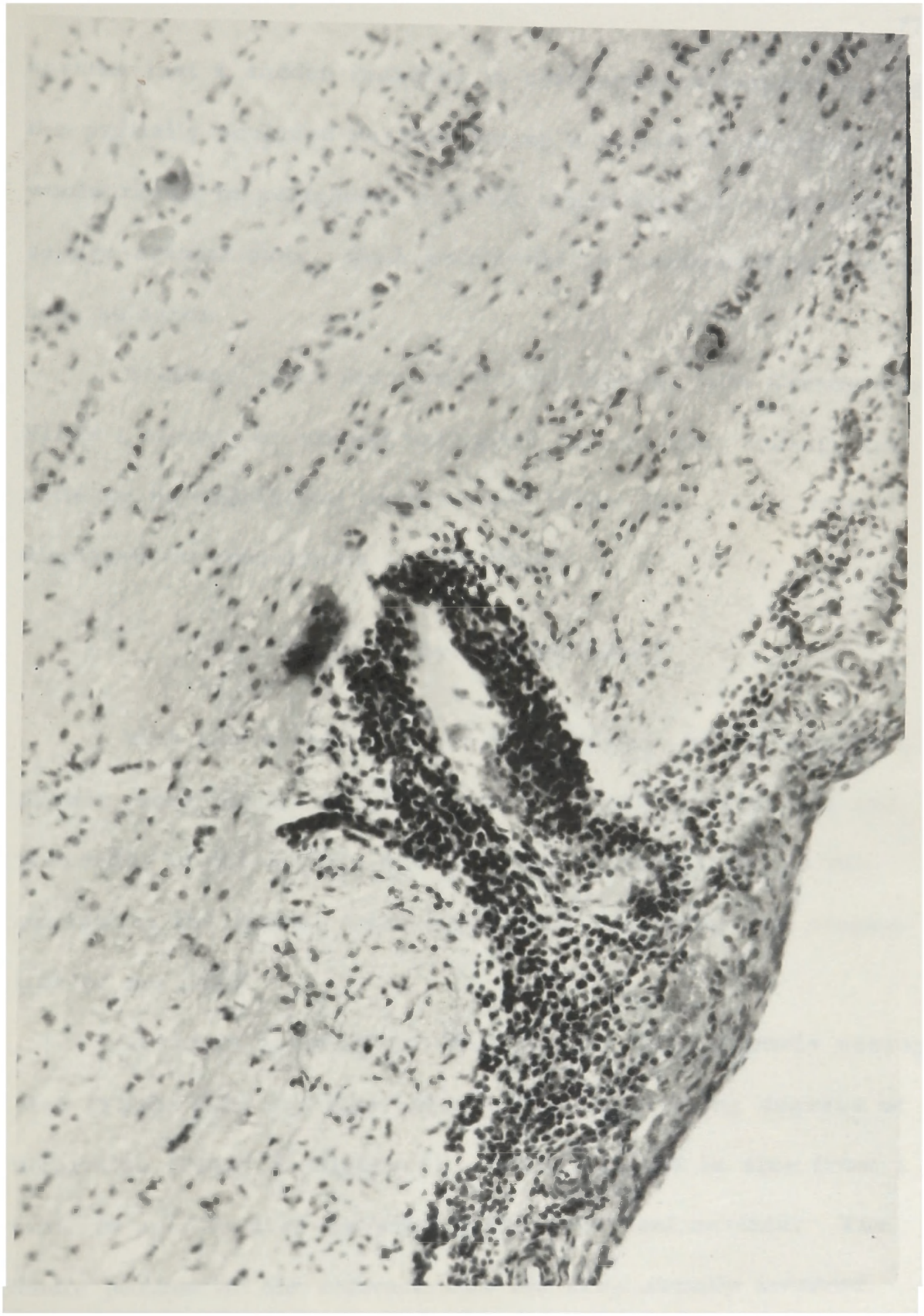
SEVERITY OF BRAIN LESIONS IN RELATION TO DAY OF DEATH OF VIRUS "A" INFECTED SWINE

Lesions	Average Day of Death	Range in Days
Severe	12.0	10-14
Moderate	14.4	10-21
Mild	12.4	7-33

It appears, however, that the partial or complete occlusion of the smaller cranial arterioles may be responsible for a dilation of larger peripheral vessels (Plate VIII) with subsequent congestion of the meningial vessels. It is reasonable to

PLATE VIII

Medulla oblongata, pig 202. Virus "A" infected. Perivascular cuffing of a peripheral vessel. This vessel appears to be somewhat distended. Hematoxylin eosin X 300.



assume that a sudden increase in blood volume pumped through the partially occluded vessels during a period of excitement would result in peripheral arterial congestion and subsequent severe cranial pain. Such pain could be manifested by convulsive seizures.

Sections taken from an animal injected with Commercial Virus I showed an almost negligible perivascular cuffing, and little or no endothelial proliferation (Plate IX). This animal displayed no convulsions at any time.

Digestive System Pathology

With few exceptions, the gross lesions of the digestive system were limited to congestion, hemorrhage, necrosis and ulceration of the gastric and intestinal mucosa. In the two exceptions the lesions were suppurative tonsillitis and congestion of the liver.

Gastritis occurred in 45.2 percent of 84 animals necropsied (Table VIII) and was characterized by varying degrees of ulceration (Plate X, Figure 1). Ulcers ranged in size from 1 mm. to one-third of the total gastric mucosal surface. The fundic portion of the stomach was the area usually involved.

PLATE IX

Medulla oblongata, pig 182. Commercial virus No. I infected. Little or no perivascular cuffing was evident in this section. Hematoxylin eosin X 730.

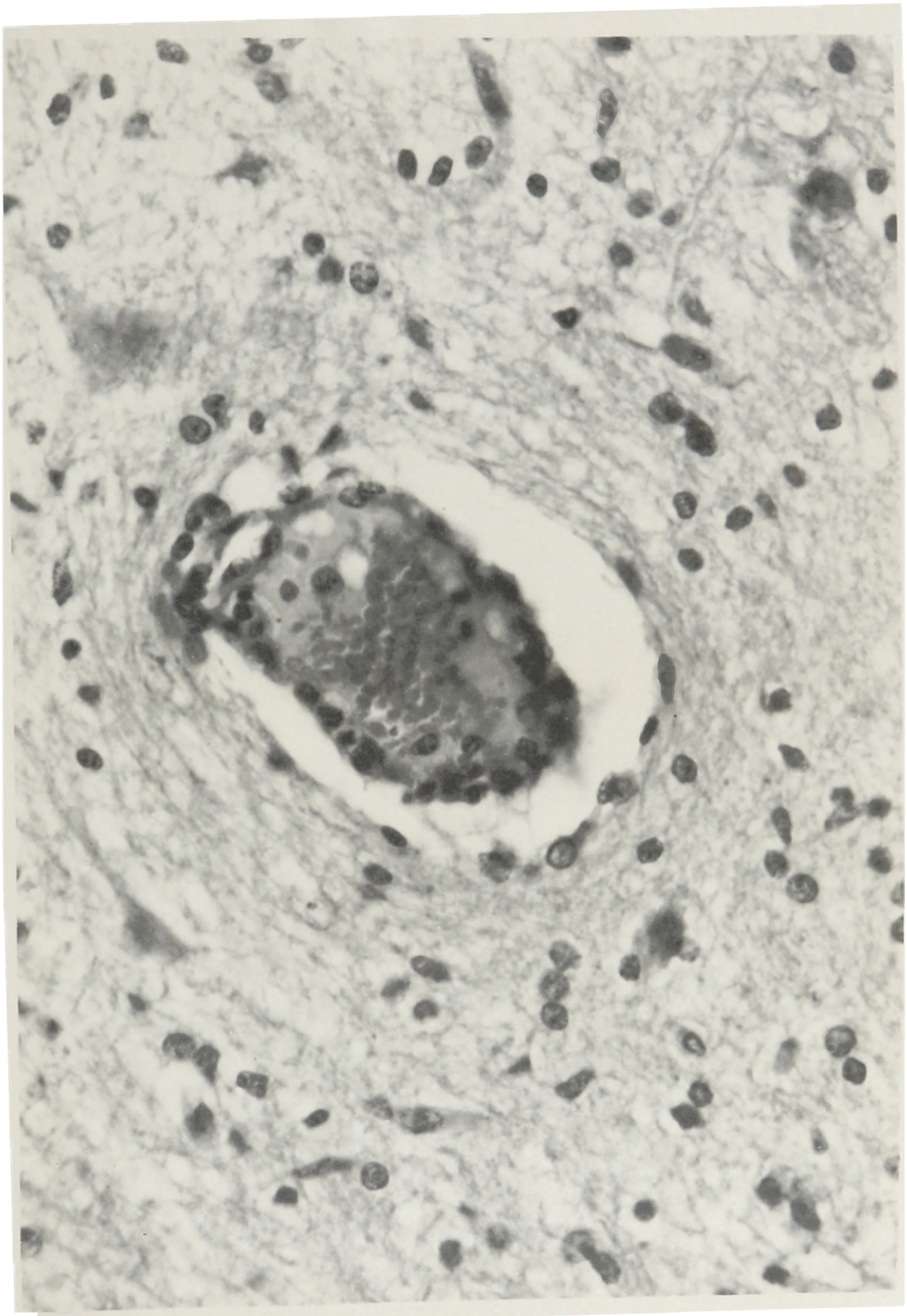


PLATE X

Figure 1. Severe ulceration of the stomach of a virus "A" infected pig.

Figure 2. Section of the colon of a virus "A" infected pig. Button ulcer formation is acute and severe.

PLATE X

Figure 1

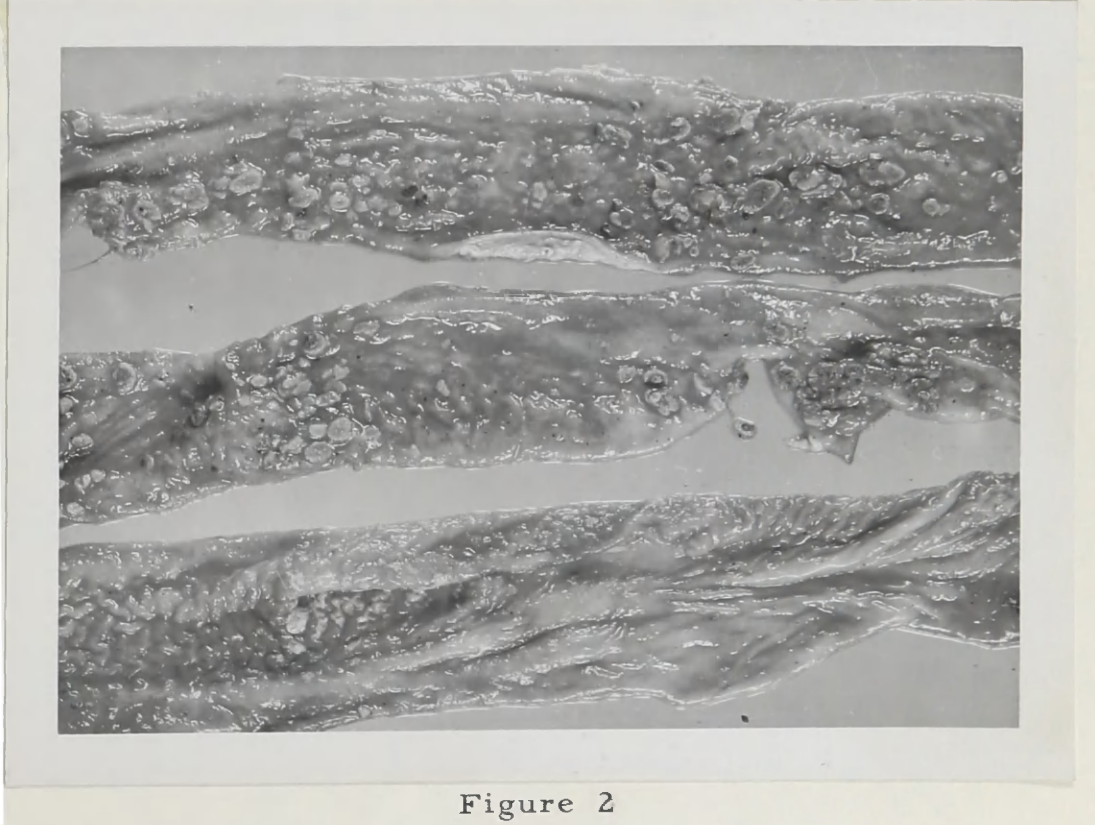


Figure 2

Little ingesta were found in the stomach at the time of necropsy. In 8.3 percent of the cases petechial hemorrhages in the mucosa were observed. In three cases (3.6 percent) there were petechial "paint brush" hemorrhages of the gastric serosa. Gastric edema occurred only twice in 84 cases.

Mild to moderate, acute catarrhal inflammation of the small intestine occurred in 15.5 percent of the cases, but was a relatively unimportant lesion.

Acute, catarrhal colitis was observed in 21.4 percent of the cases, and diffuse necrotic colitis and cecitis were seen in 16.8 percent. All necrotic enteritis cases were acute to subacute. Button ulceration of the colon was evident in 20.2 percent of the cases. Ulcers were generally in an early stage of development (Plate X, Figure 2, and Plate XI, Figures 1 and 2). In a few instances they were quite acute. Nutritional enteritis type lesions were observed in three cases. The lesions were characterized by fecal plaques adhered to the intestinal mucosa by mucous strands as described by Dunne et al. (1949). Only 10.7 percent of the animals showed colonic petechiation. In an additional three instances there was hemorrhage into the intestinal lumen.

PLATE XI

Figure 1. Same intestine as in Plate X, Figure 2, showing the congestion of the colonic mucosa that accompanied the ulcer formation.

Figure 2. Very acute generalized necrotic enteritis of the cecum with marked congestion.

PLATE XI

Figure 1

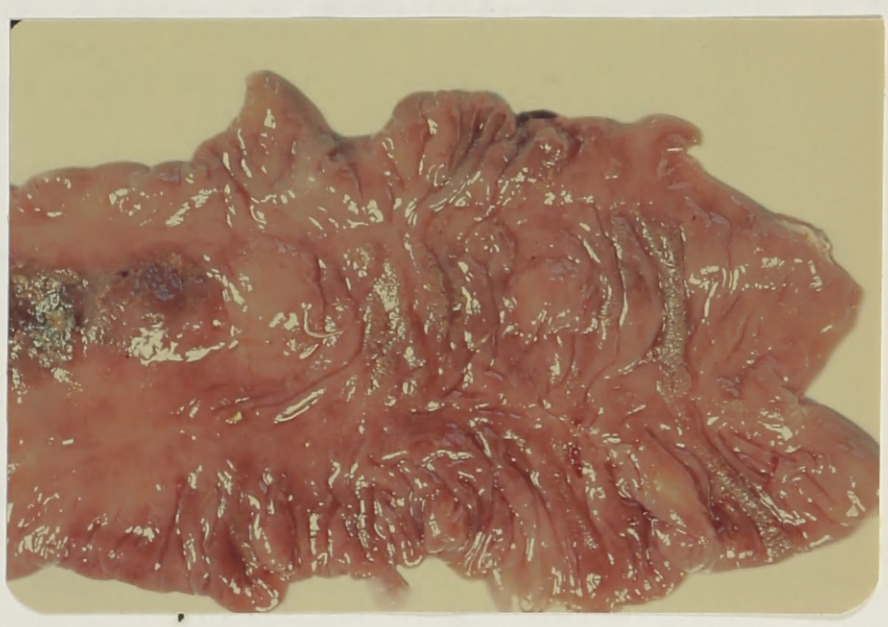


Figure 2

Microscopically the digestive tract lesions were characterized by endothelial degeneration, necrosis of the mucosa and cellular infiltration. Several very small developing button ulcers were secured for tissue section. When observed microscopically these lesions were characterized by coagulation necrosis of a small mucosal area separated from healthy tissue by a line of invading leucocytes (Plates VII through XVI). The fact that leucocytes were absent from the necrosed tissue but were massed in a line at its periphery indicated that the area was an infarct. This would suggest that the button ulcer was formed as the result of the intestinal infarction. Further evidence of this is the presence of partially occluded arteries in the colonic submucosa. Endothelial proliferation and hydropic degeneration of the endothelial cells appear to completely occlude the vessels in some cases (Plate XIX). Leucocytic margination at the intima of the vessels (Plates XIX, XXI) also promotes the occlusion of the vessels. These observations were also made in a few cases of gastric ulceration (Plates XX and XXI). Again, only in the very acute stage of ulceration could the infarct be recognized.

PLATE XII

Button ulcer of colon in an early stage of formation. Note the area of coagulation necrosis in the mucosa separated from healthy tissues by a wide dark band of leucocytes, suggesting the formation of an infarct. Hematoxylin eosin X 100.

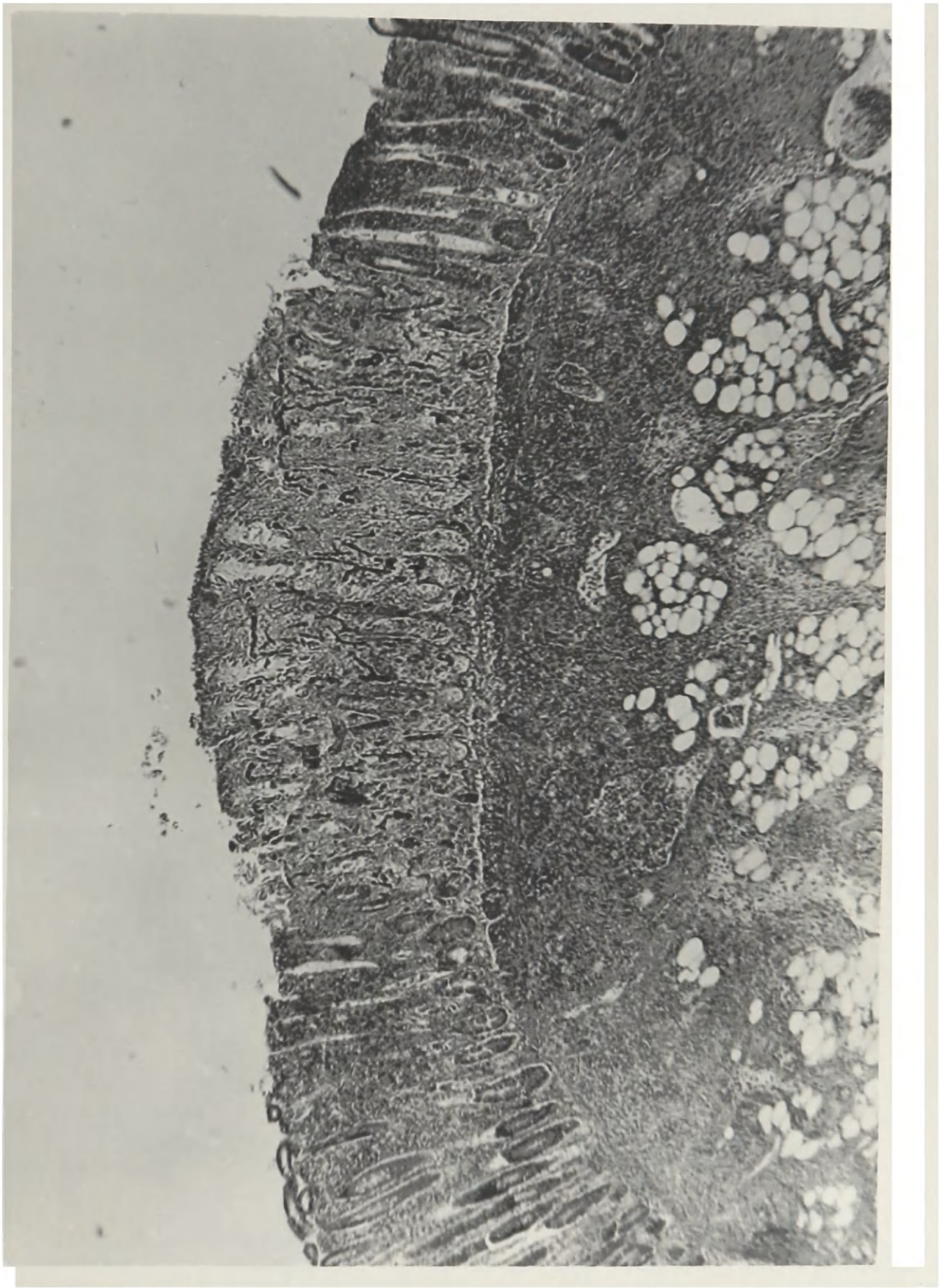


PLATE XIII

Higher magnification of the edge of the ulcer shown in Plate XII illustrating in more detail the coagulation necrosis and the healthy tissue separated by a band of infiltrating leucocytes. Hematoxylin eosin X 300.

PLATE XIV

Button ulcer of colon at a slightly later stage showing a more marked infiltration of leucocytes at the periphery of the infarcted area. Hematoxylin eosin X 100.

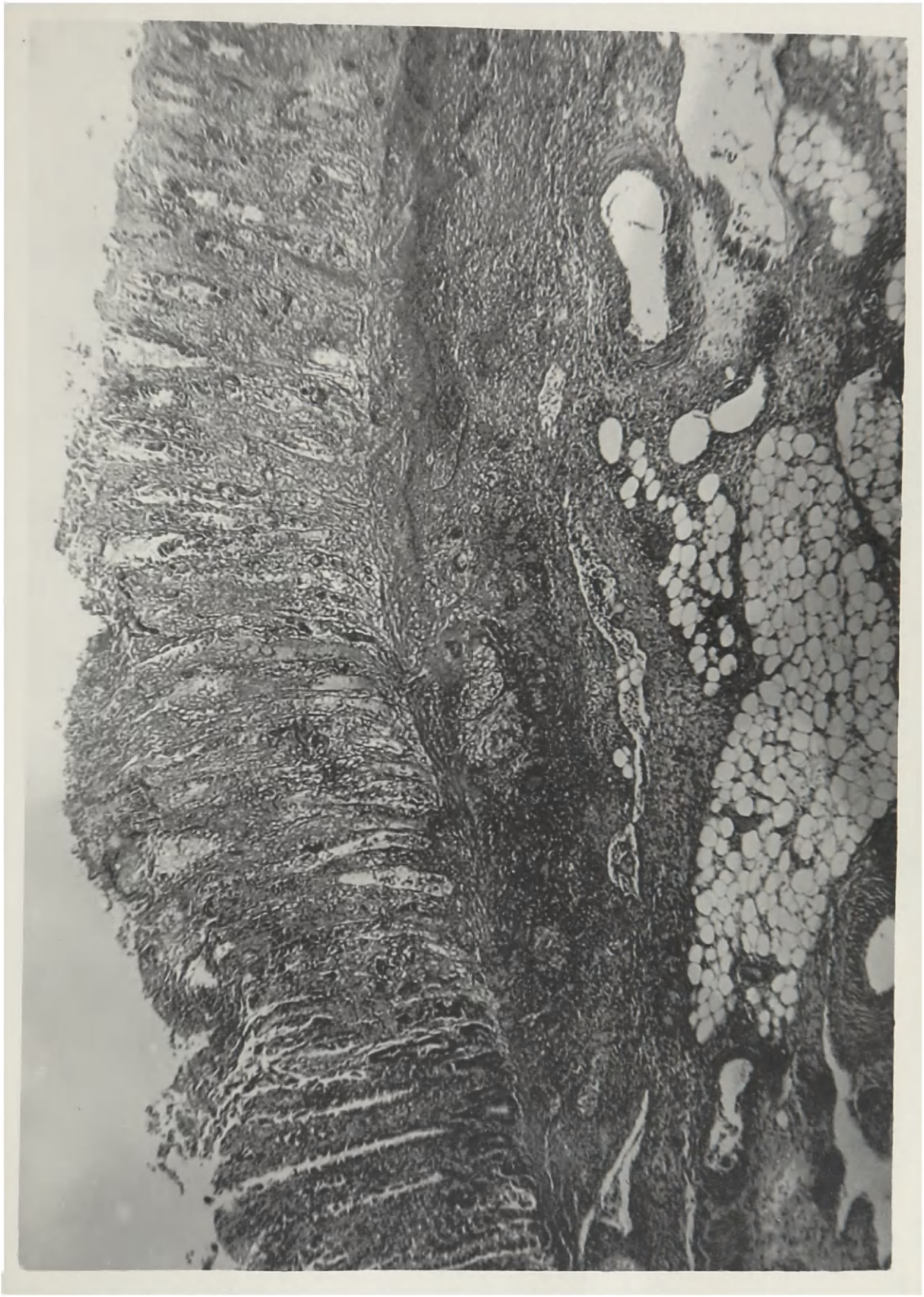


PLATE XV

High magnification of an area at the base of the infarcted mucosa shown in Plate XIV illustrating the indefinite cell structure and the absence of leucocytes in the mucosa. The submucosa, however, abounds in neutrophiles, lymphocytes and some mononuclear phagocytes. Hematoxylin eosin X 700.

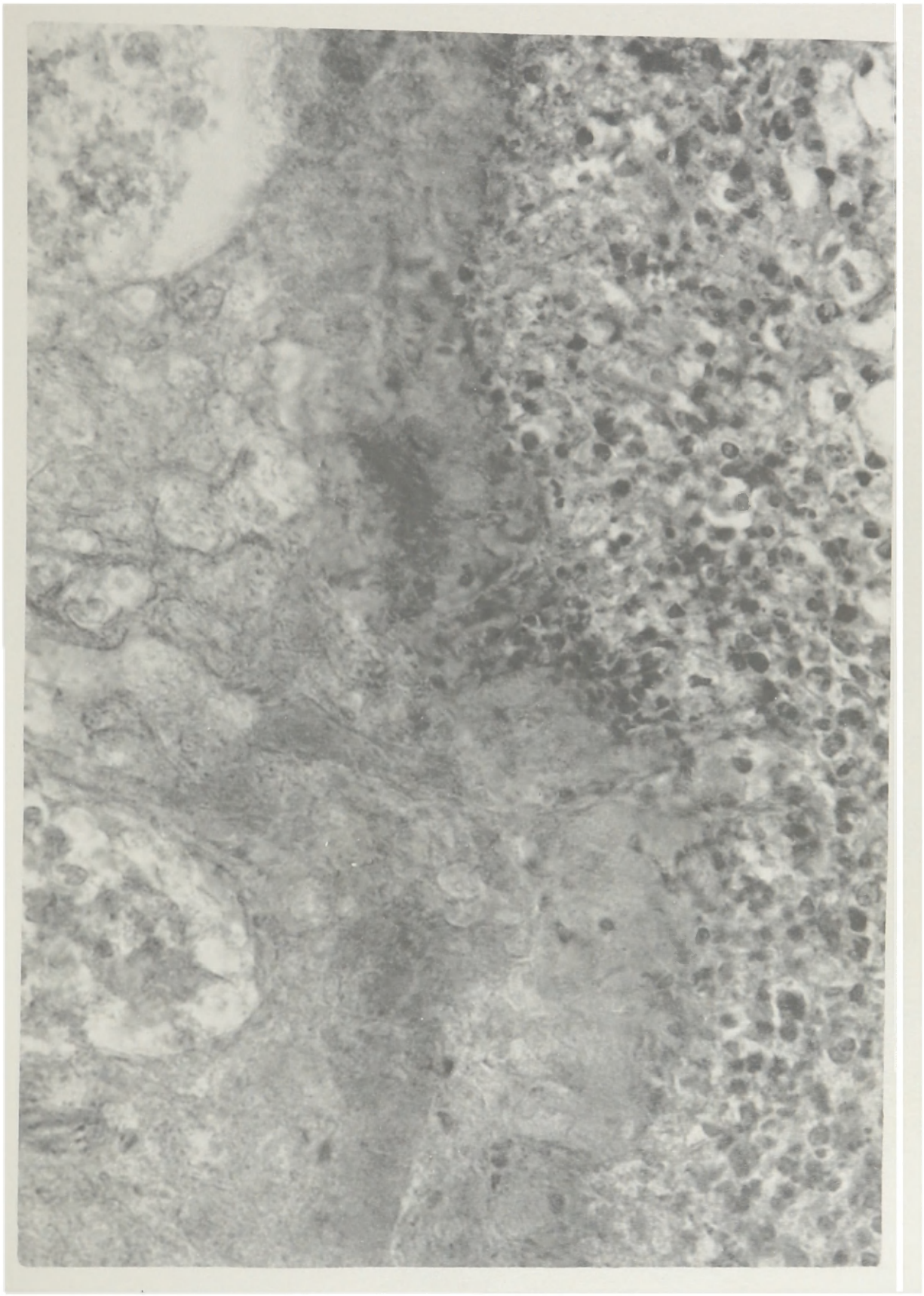


PLATE XVI

Button ulcer of colon in advanced stage. The necrosed mucosa is forced upward by the mass of infiltrating leucocytes. Note the faint outlines of intestinal villi at "A" in the sloughing mucosa with the dark line of phagocytic leucocytes at the base of the area at "B." Note also at "C," the upward curvature of the muscularis mucosa. At "D" observe the partially occluded blood vessels. Many smaller vessels appear completely occluded. Hematoxylin eosin X 100.

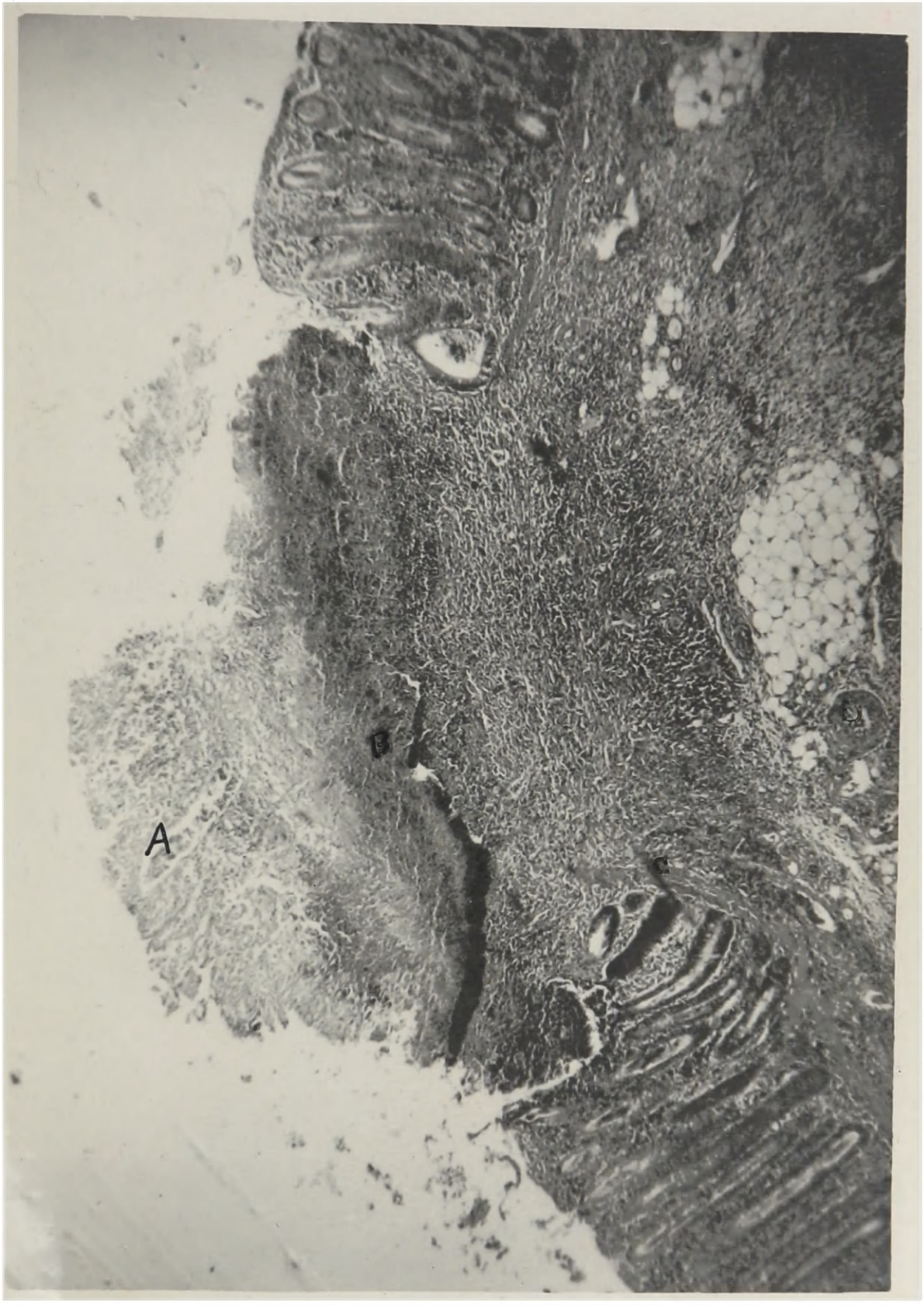

A

PLATE XVI

Button ulcer of colon in advanced stage. The necrosed mucosa is forced upward by the mass of infiltrating leucocytes. Note the faint outlines of intestinal villi at "A" in the sloughing mucosa with the dark line of phagocytic leucocytes at the base of the area at "B." Note also at "C," the upward curvature of the muscularis mucosa. At "D" observe the partially occluded blood vessels. Many smaller vessels appear completely occluded. Hematoxylin eosin X 100.

A

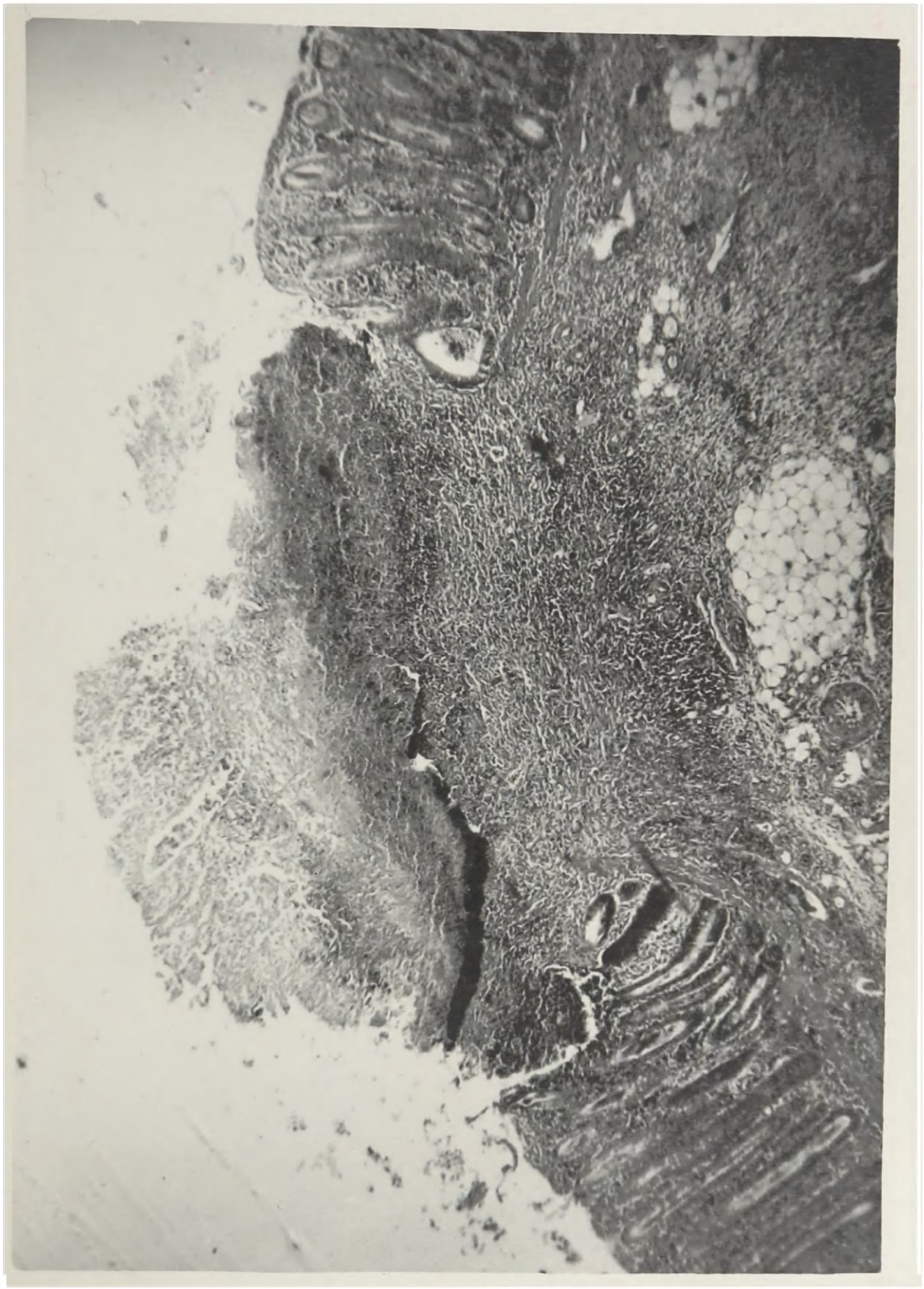


PLATE XVII

Ulcerating stage of a colonic infarction following the sloughing of the mucosa. Coagulation necrosis of a portion of the infarcted tissue and the line of infiltrating leucocytes is still visible. Hematoxylin eosin X 100.

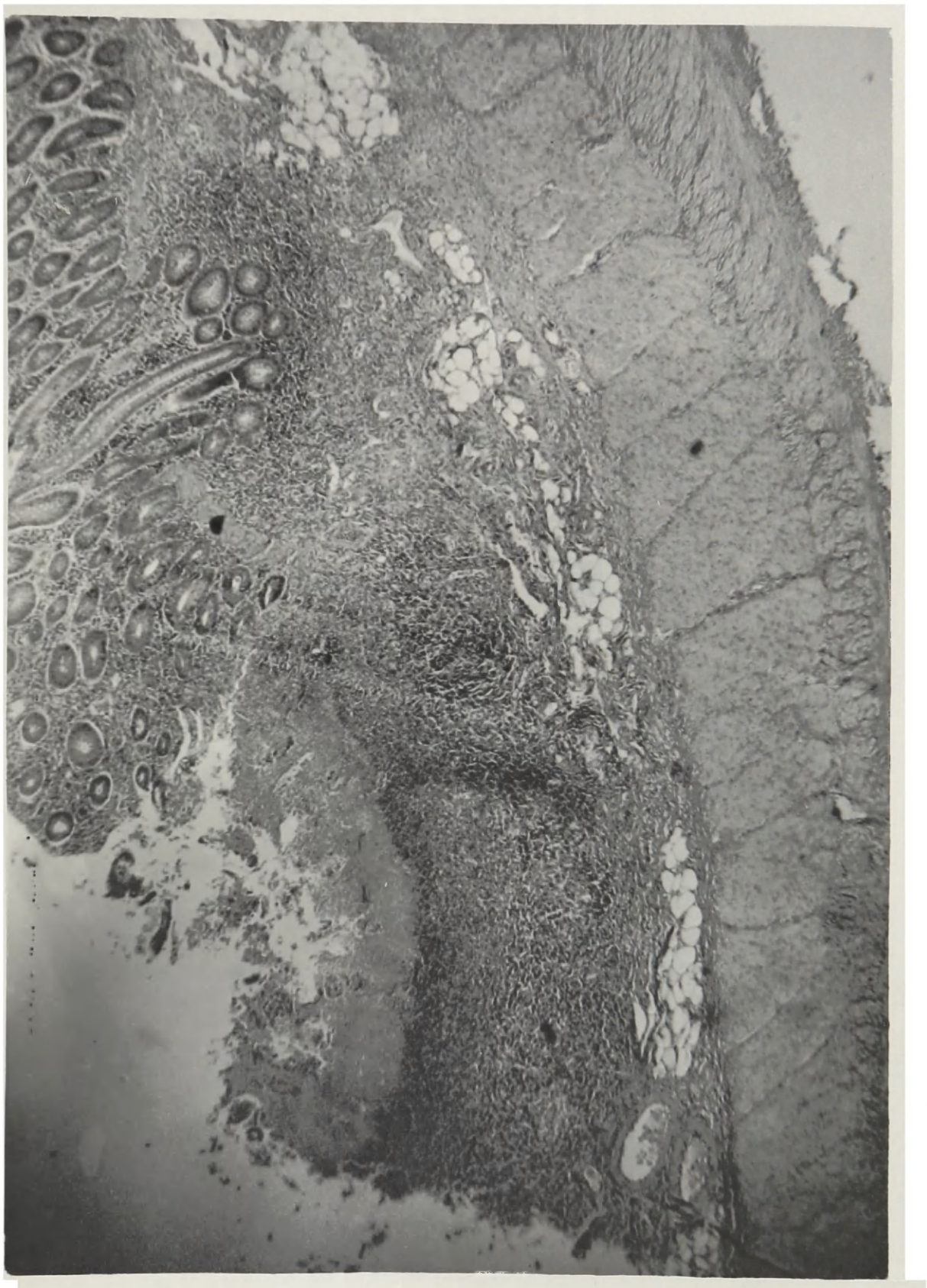


PLATE XVIII

A late stage of ulceration showing tissue reaction to invading intestinal bacteria following the sloughing of the mucosa. Hematoxylin eosin X 100.

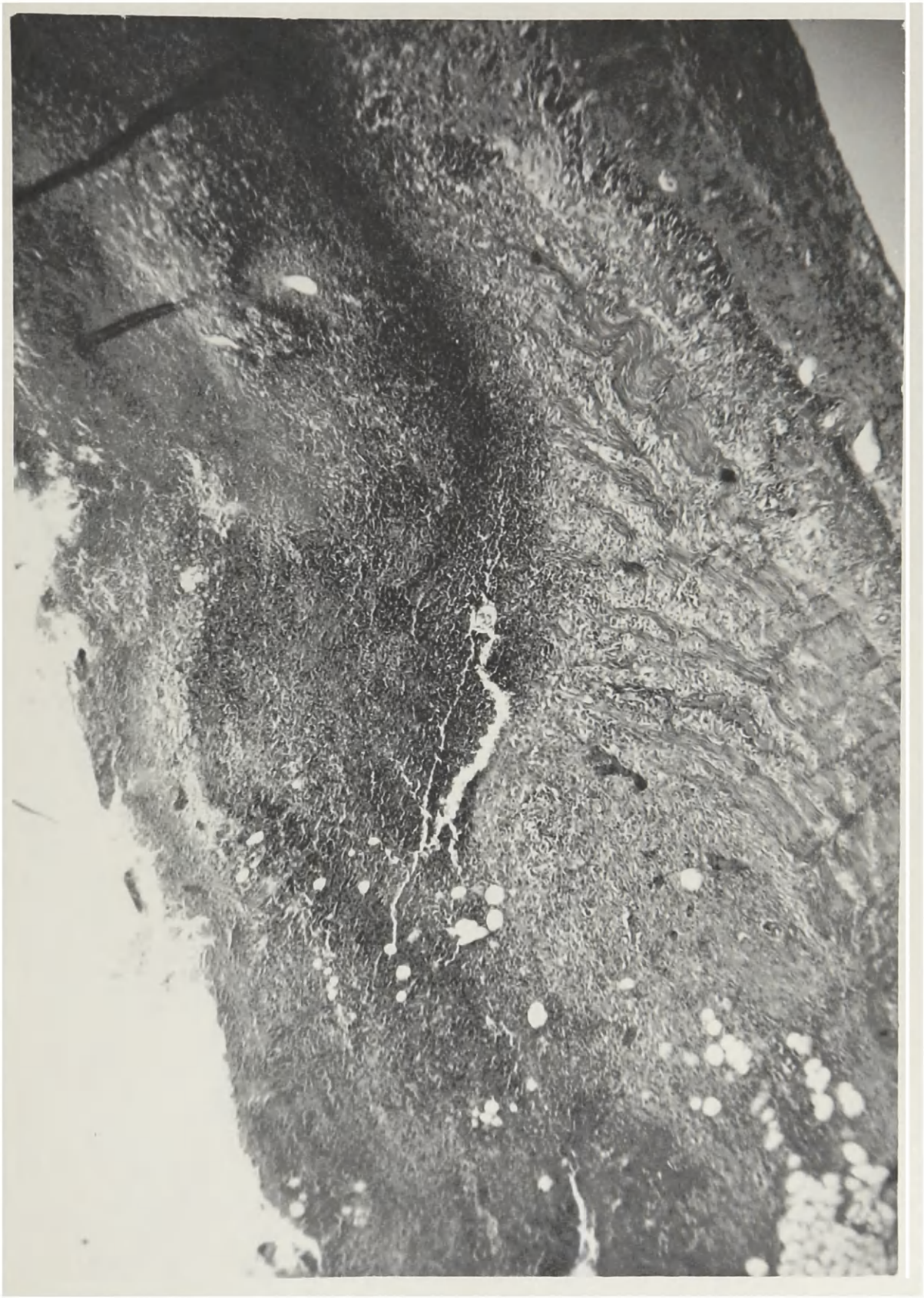


PLATE XIX

An almost completely occluded arteriole observed at the base of the infarct shown in Plate XVI. Note the hydropic degeneration of the endothelial cells and the margination of the few leucocytes present. Hematoxylin eosin X 800.

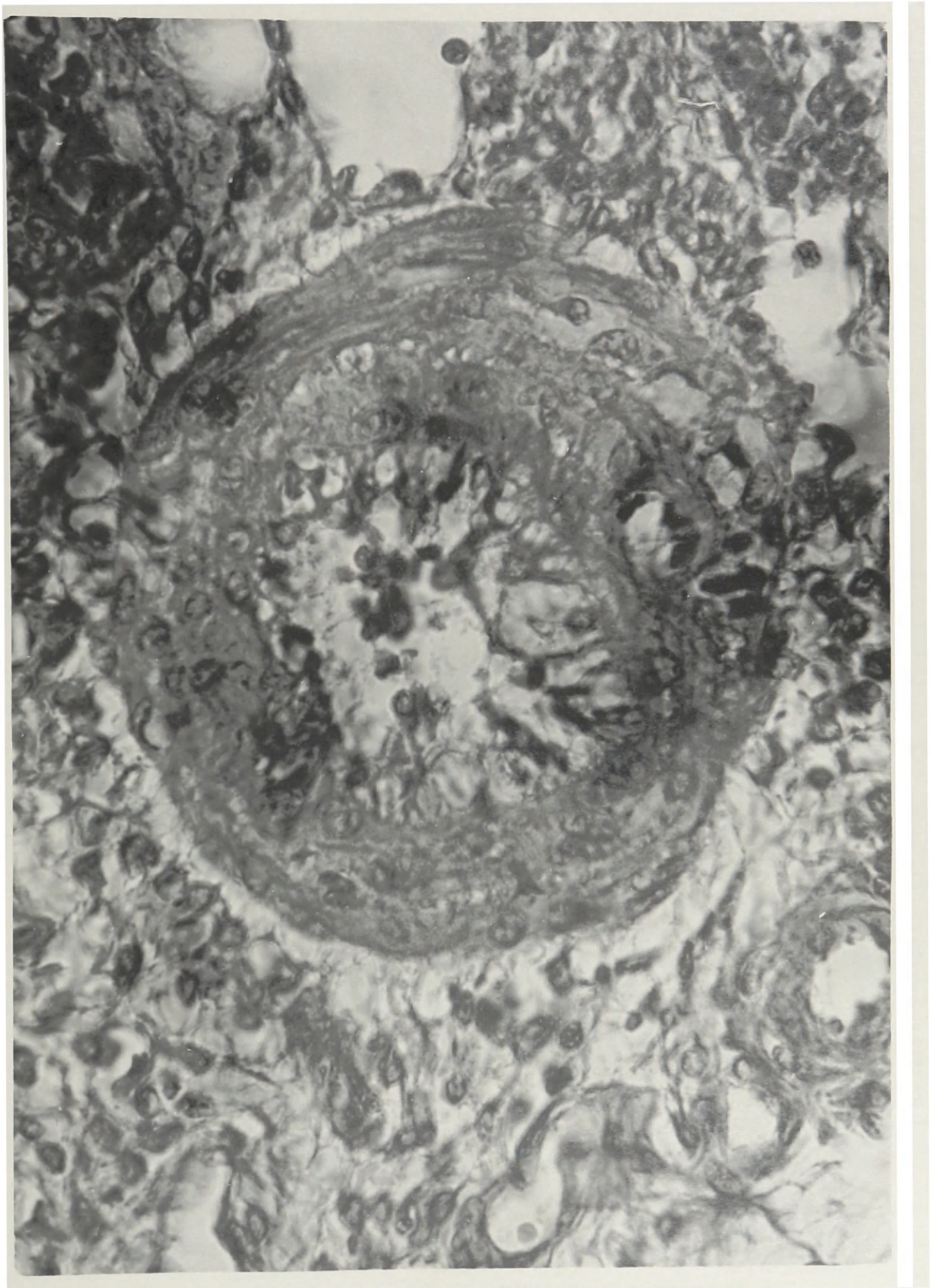


PLATE XX

Preulceration stage of a gastric infarct showing a line of leucocytes bordering an area of coagulation necrosis. Hematoxylin eosin X 100.

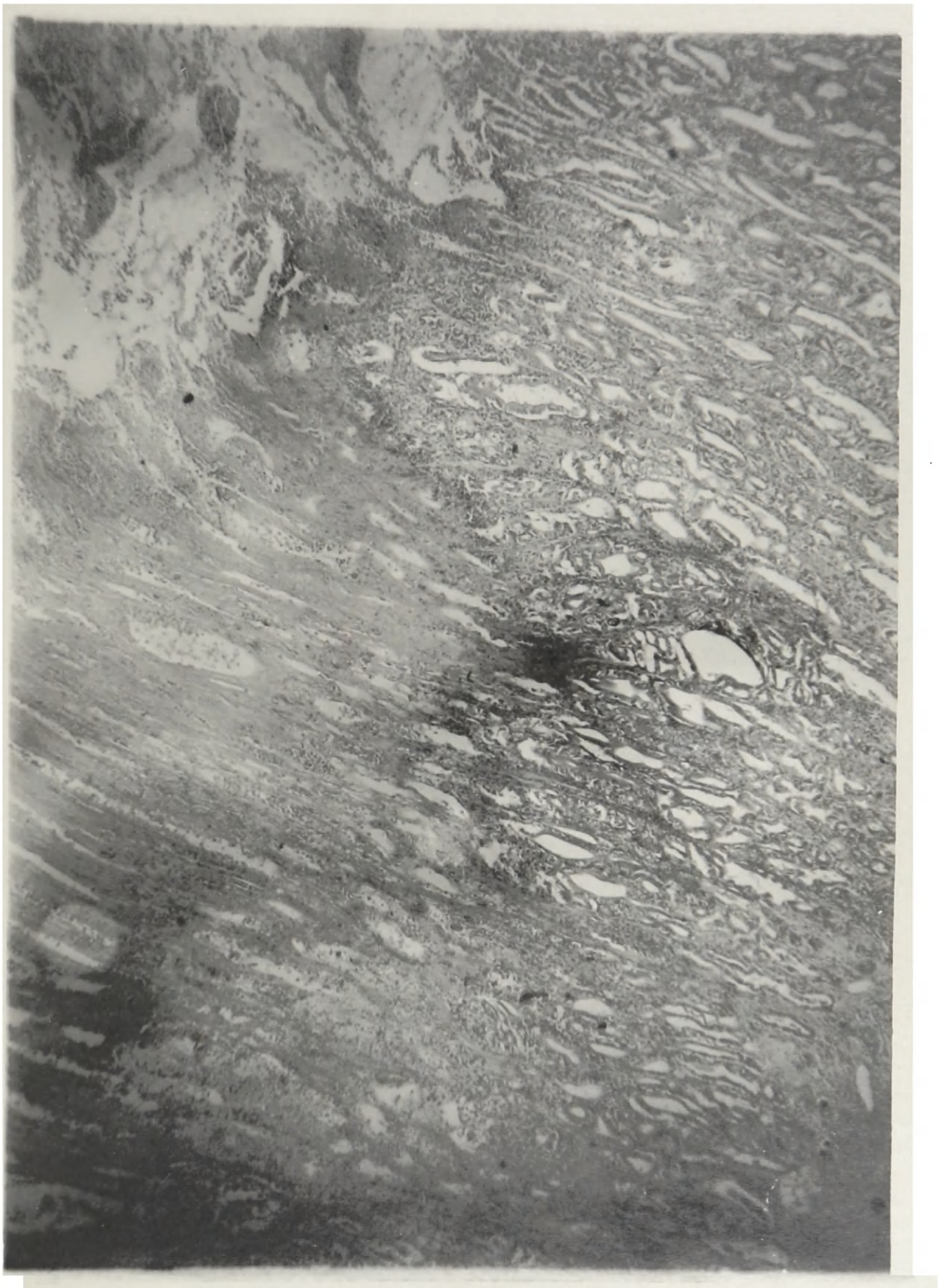
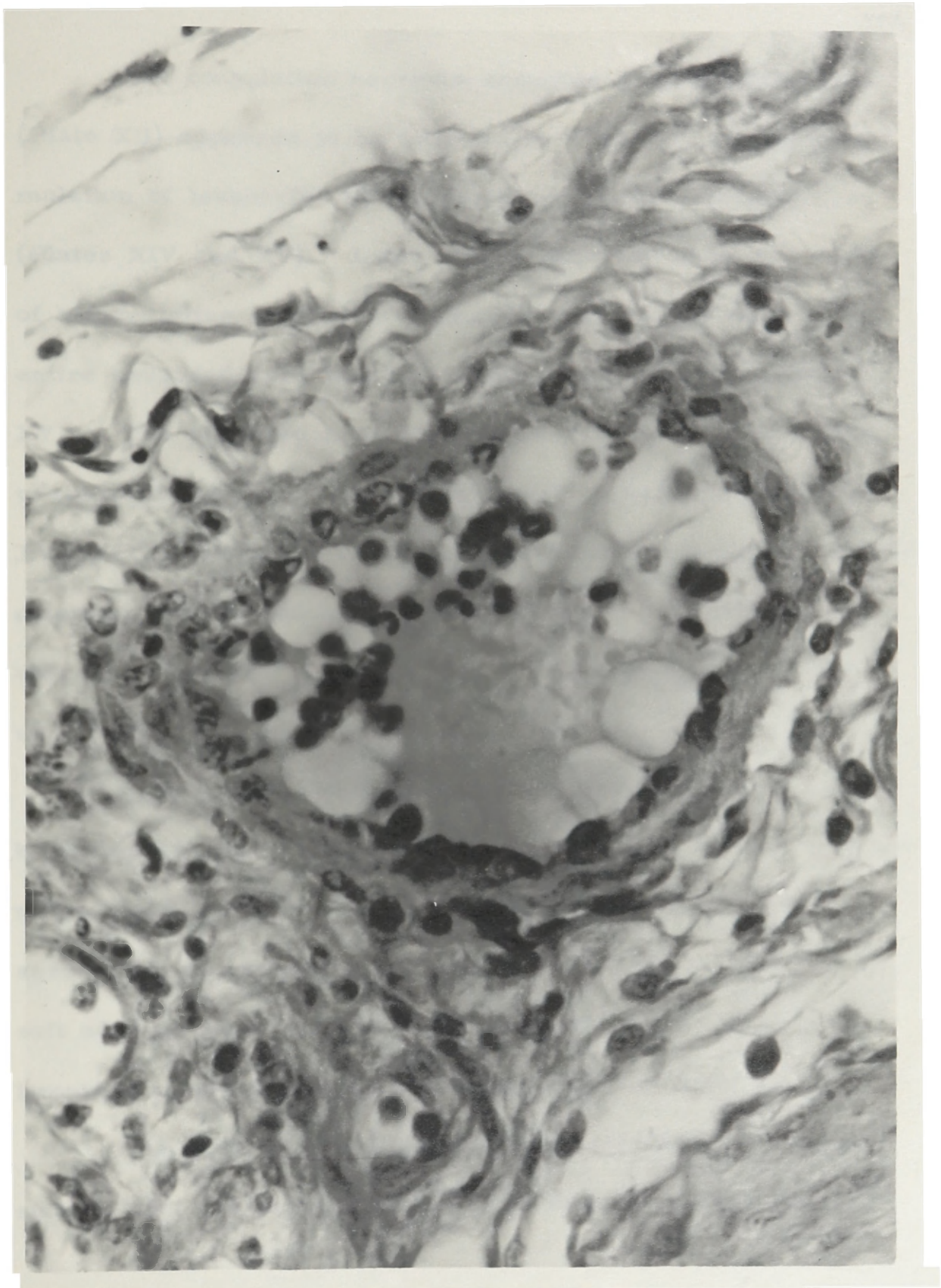


PLATE XXI

Venule at the base of the infarct shown in Plate XX. Note the hydropic degeneration of the endothelial cells and the margination of leucocytes. Hematoxylin eosin X 850.



The coagulation necrosis resulting from the infarction (Plate XII) appeared to be followed very shortly by the accumulation of leucocytes at the periphery of the infarcted area (Plates XIV and XV). Later stages observed showed a mass of accumulating leucocytes primarily neutrophiles forcing the entire necrosed area into the lumen of the intestine (Plate XVI). The necrosed area was sloughed in older lesions and caseation necrosis of the leucocytic mass was evident (Plate XVII). The caseation necrosis in some cases appeared to have extended into the submucosa with the effect of producing an abscess. In other cases healing from the periphery of the ulcer seemed to be in progress.

Well established button ulcers appeared at first to have been formed over nodules of lymphoid tissue. It later seemed more likely, however, that in view of the afore-mentioned observations, the presence of the lymphatic nodules was the result of hyperplasia of one of the minutely small islands of lymphoid tissue distributed throughout the intestinal wall.

The general or diffuse necrotic enteritis in a few instances was characterized by the histological appearance of

bacterial infection (Plate XXII) although no salmonellae were isolated from the lesions.

Rickets Associated With Hog Cholera

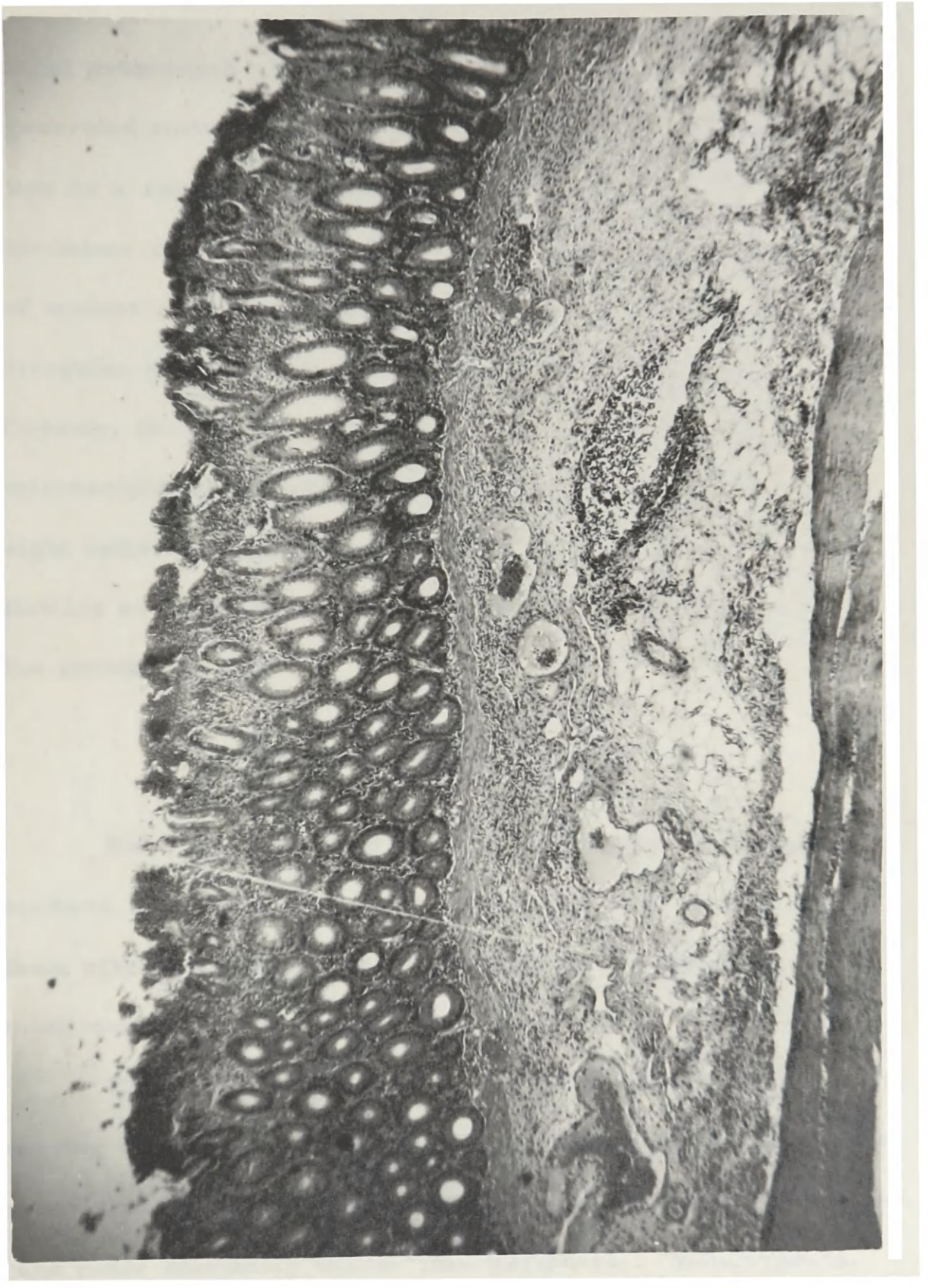
There was a definite enlargement of the ends of the ribs and an apparent increase in the amount of red bone marrow. The epiphyseal line of the costochondral junction appeared broadened and irregular, while the bony matrix appeared to have decreased. Clinically, during the late stages of the disease, marked knuckling of the front legs was noticed in some instances. One animal, after developing severe knuckling during the initial stages, later developed a posterior paresis. For several days before death the animal dragged about by the front legs.

Calcium and phosphorus determinations of the blood revealed that calcium was present at an average level of 9.9 mgm. percent in two animals and phosphorus at a level of 5.13 mgm. percent in one animal.

Microscopic observations of bone lesions of animals suffering from hog cholera verified the gross observations of a wide epiphyseal line and a decreased calcified matrix. The

PLATE XXII

Area of acute colitis attributed to bacterial infection. Note the concentration of bacteria and cellular elements on the surface of the mucosa and the accumulation of neutrophiles in the distended veins and lymph vessels. Hematoxylin eosin X 100.



most pronounced change was the increased number of undegenerated mature cartilage cells and the absence of calcification in a relatively wide area between the bone marrow and the immature cartilage cell area (Plate XXIII). The large number of mature cartilage cells was manifested grossly by a wide irregular white line at the junction of the bone and cartilage. Grossly, the normal white line is narrow and straight, and microscopically the normal mature cell layer is only six to eight cells in width (Plate XXIV). The fine spicules of ossification are evident in the matrix below the epiphyseal line of the normal section.

Pathology of Other Tissues

Since the lesions of most of the organs conformed to textbook descriptions of the disease, little will be said about them other than to stress the severity of lesions observed in many cases.

The lymph nodes were congested or showed evidence of varying degrees of hemorrhage in more than 96 percent of the cases studied. Hemorrhage generally was moderate to severe, and more commonly diffuse than peripheral. Both types of

PLATE XXIII

Acute ricketic lesion at the costochondral junction of the eighth rib. Note the irregularity of the epiphyseal line and the long columns of mature cartilage cells which give great width to the white line observed grossly. Hematoxylin stain X 175.

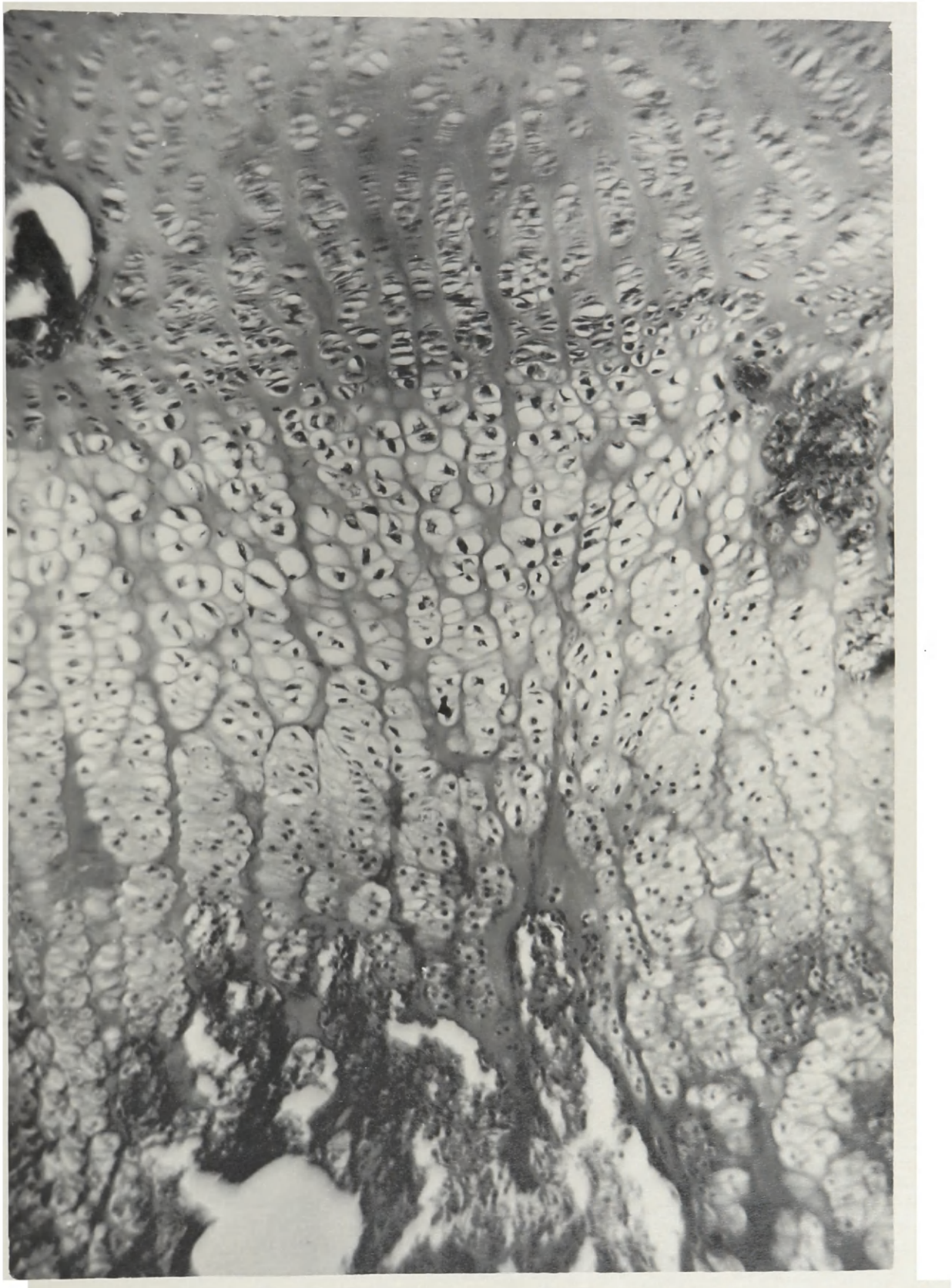
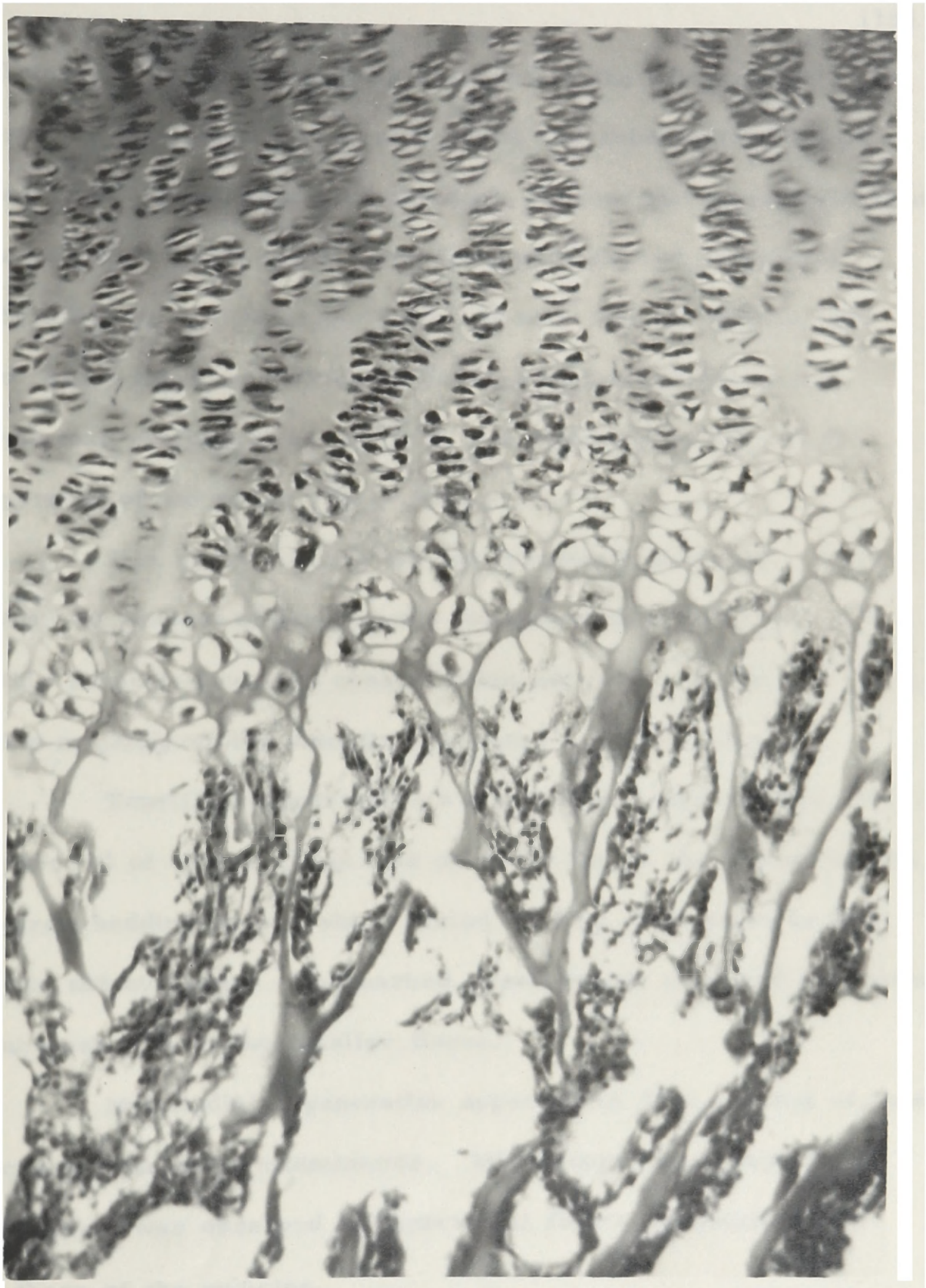


PLATE XXIV

A normal section from costochondral junction of the eighth rib. Observe the regular thin line formed by short columns of mature cartilage cells. Note also the fine spicules of mature bone in the area below the junction. Hematoxylin eosin X 200.



hemorrhage were frequently observed in the same animal. In such cases, the cervical, pharyngeal and submaxillary lymph nodes displayed diffuse hemorrhage whereas the mesenteric and associated lymph nodes more frequently showed peripheral hemorrhage. It was interesting to note that the diffuse type of hemorrhage was recorded in 71.4 percent of the cases, whereas peripheral hemorrhage was observed in only 47.6 percent of the cases (Table VIII).

Skin erythema was not too frequently observed. Less than 30 percent of the animals showed definite erythema. In this respect, however, consideration was given to the fact that the majority of the animals inoculated had black skins.

Tonsillitis occurred in a surprisingly high number (38.1 percent) of cases. This was partially due to the use of wheat straw bedding over a short period of time when other bedding was not available. The barbed wheat beards produced pronounced abscessation in the tonsilar tissue.

Myocardial degeneration appeared in 35.7 percent of the cases upon gross examination. Microscopically, coagulation necrosis was observed in myocardial fibers, particularly in those of the auricles.

Bronchopneumonia was observed in 58.3 percent of the necropsied animals. Approximately three-fifths of this number were mild in character. Only seven of the 49 pneumonia cases were of a severe nature. In many instances, the lung was unilaterally affected, indicating a hypostatic type of pneumonia. In one instance a very severe acute type of local necrosis was observed. Areas of the lung two to four cm. in diameter were completely lysed as if the area had been necrotic and sloughed away (Plate XXVI). Psudomonas aeruginosa was isolated from the heart blood of this animal and may have played an important part in the development of the lesions. Pulmonary ecchymosis was observed in 15.7 percent of the necropsies and more than half of these were severe in nature.

Lesions of the kidney provided some of the more spectacular changes observed in animals infected with virus "A." Hemorrhages of the cortex varied from mild petechiation to severe ecchymosis. In some instances mild petechiation of the cortex was associated with severe diffuse hemorrhage of the pyramids (Plate XXVII, Figure 2). In other cases the cortex would show severe petechiation with less severe hemorrhage of the pyramids (Plate XXVII, Figure 1). The most

PLATE XXV

Acute myocardial degeneration of an auricular area. Note the cloudy swelling in the light staining band of heart muscle. Hematoxylin eosin X 465.

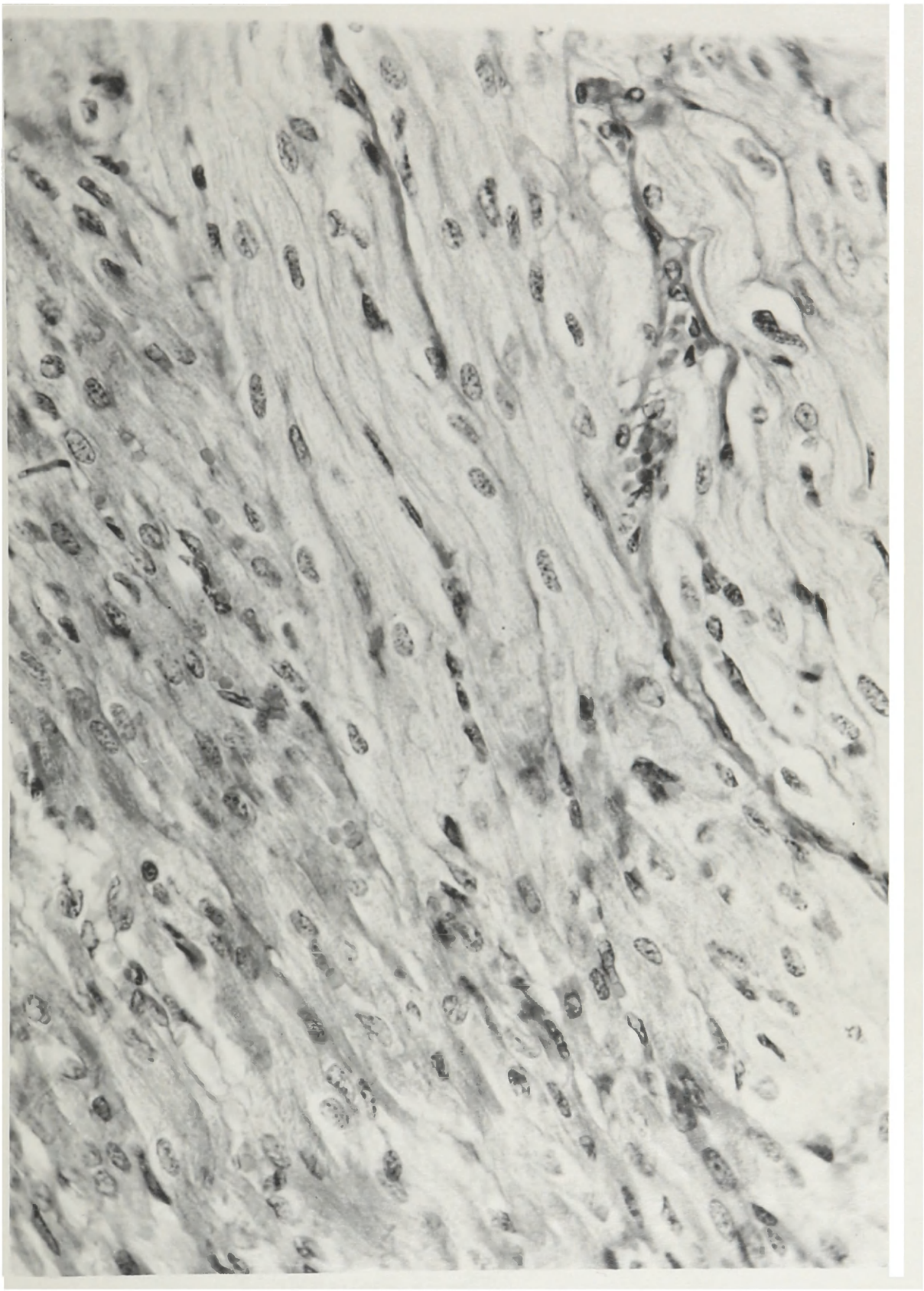


PLATE XXVI

The lung of a virus "A" infected pig, from which Pseudomonas aeruginosa was isolated. Note cavities from which tissue had been lysed.

PLATE XXVI

PLATE XXVII

Figure 1. Severe petechiation of the cortex of a virus "A" infected kidney with only mild hemorrhage of the medulla.

Figure 2. Mild cortical petechiation with severe medullary hemorrhage.

PLATE XXVII

Figure 1

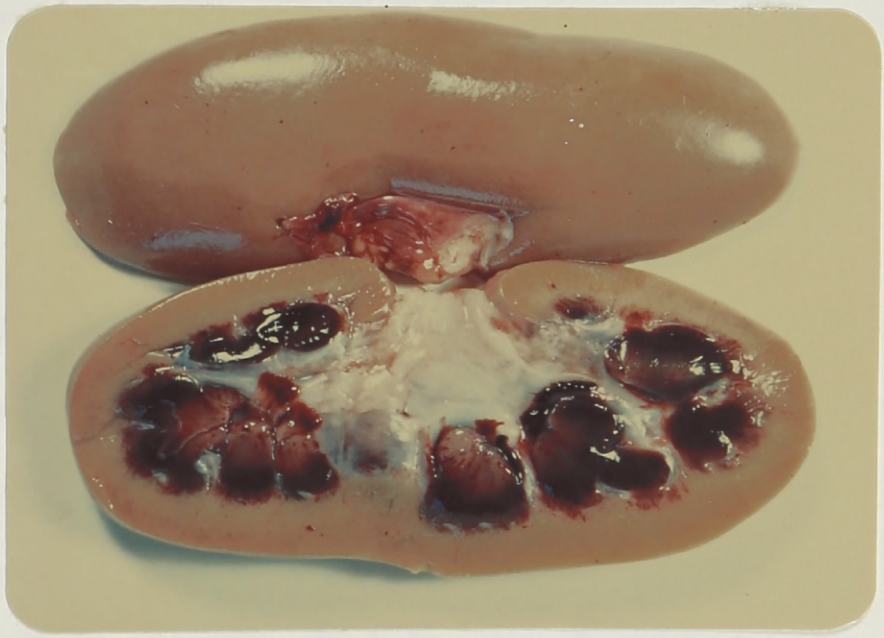


Figure 2

pronounced lesions were in those cases in which severe ecchymotic "turkey egg" lesions appeared in the cortex of the kidney and diffuse hemorrhage was present in the pyramids (Plate XXVIII, Figure 1). Hemorrhage of the pyramids was observed in 28.6 percent of the postmortem examinations.

Although a thorough microscopic examination of kidney sections was not undertaken, the few sections examined revealed extensive hemorrhage into the interstitial tissue. The cells of the tubules were undergoing coagulation necrosis with some karyolysis already evident (Plate XXIX).

Splenic infarction was quite common and rather severe in nature. Multiple infarctions were the rule occurring both on the borders and in the parenchyma (Plate XXVIII, Figure 2).

Purity Tests

Using the technics described under "Methods" bacteriological examinations revealed no S. choleraesuis in heart blood, brain, or intestinal tissues. No streptococci were isolated from brains of the animals even though particular attention was given to those animals which had shown convulsions.

PLATE XXVIII

Figure 1. Severe cortical and medullary hemorrhage of the kidney from a virus "A" infected pig.

Figure 2. Multiple infarction of the spleen from a virus "A" infected pig.

PLATE XXVIII

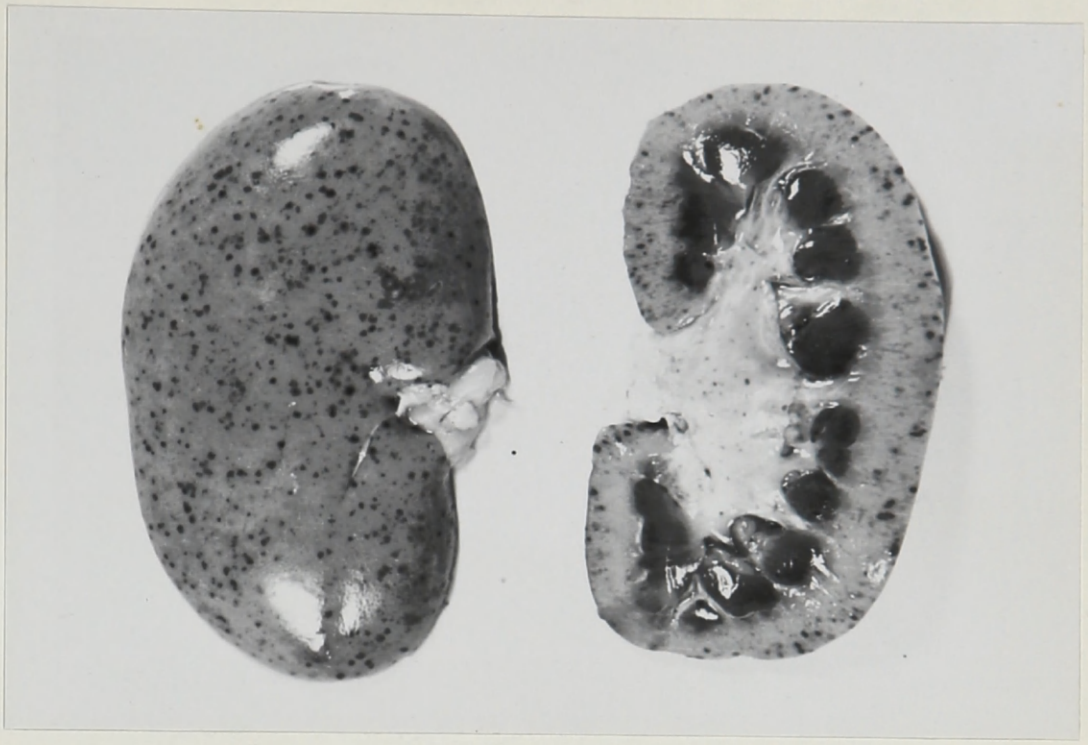


Figure 1

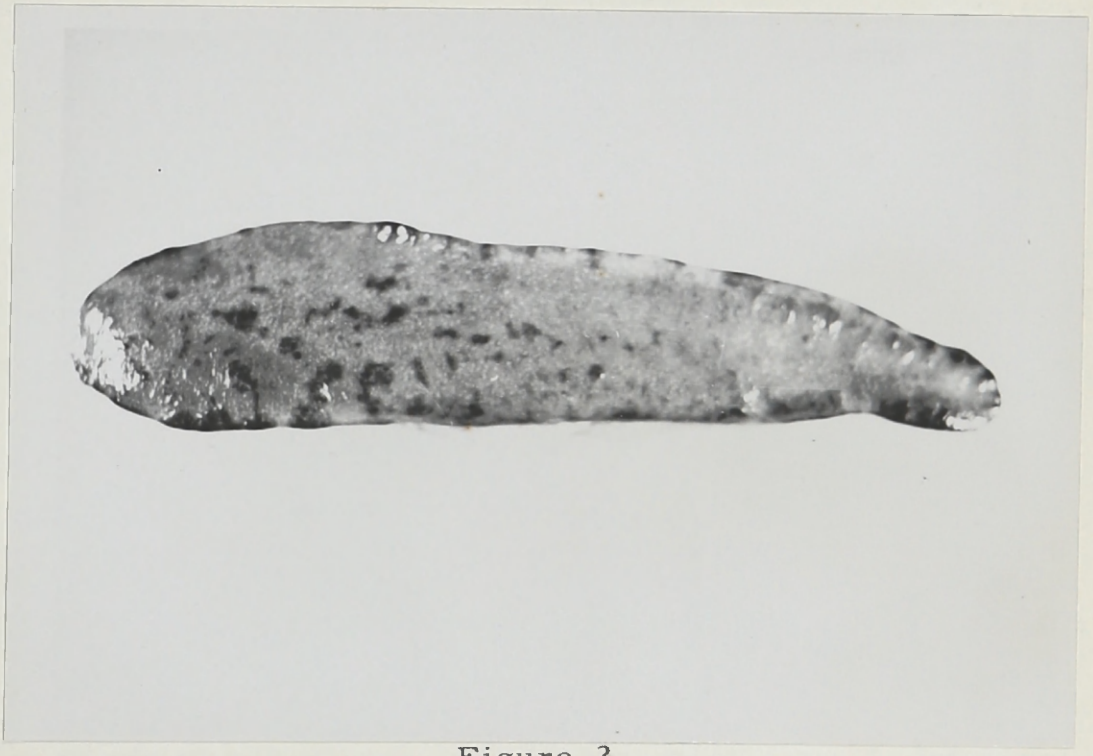
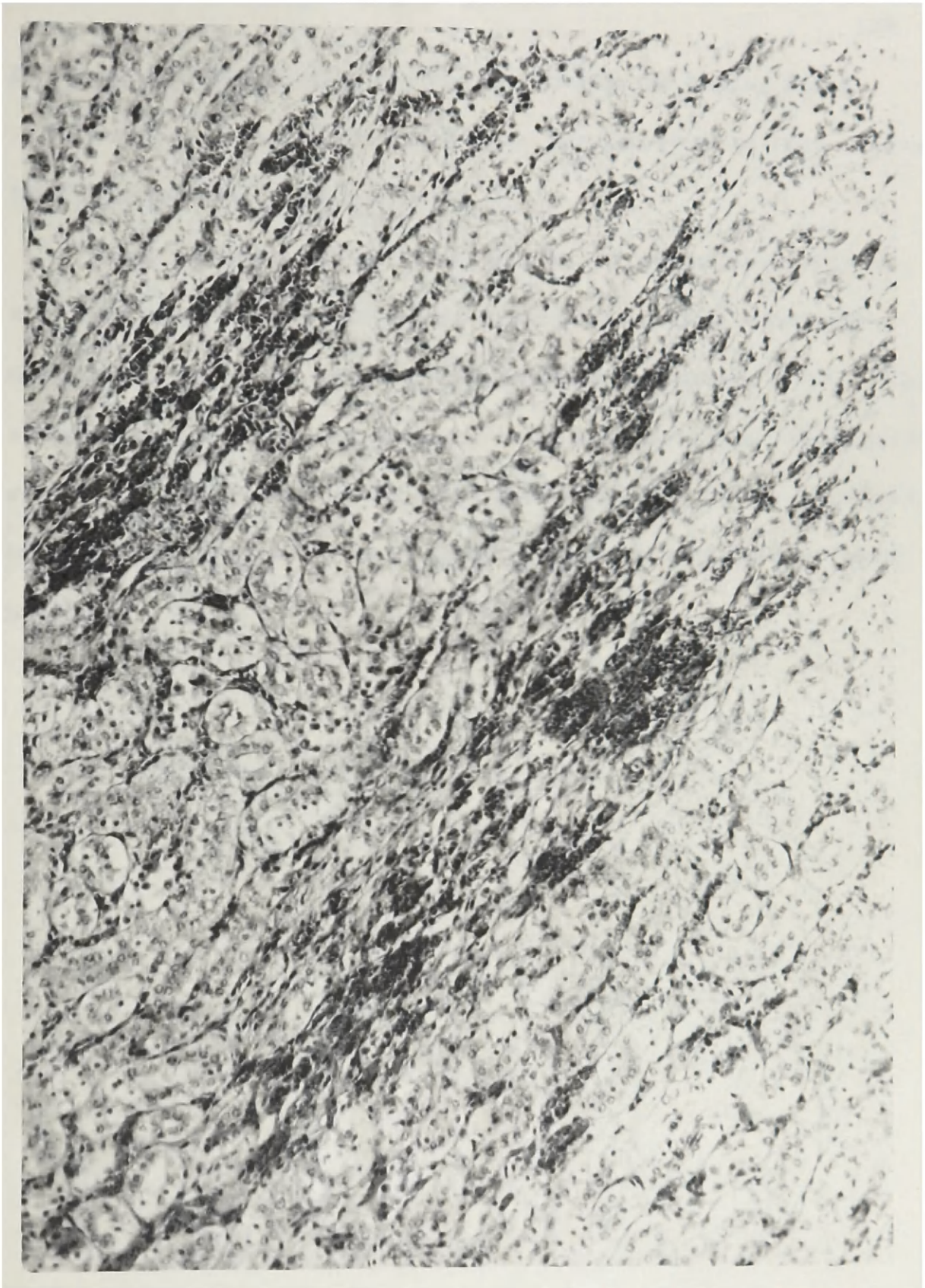


Figure 2

PLATE XXIX

The inner zone of the medulla of an infected kidney showing severe interstitial hemorrhage and degenerative changes of the tubules. Hematoxylin eosin X 300.



Pseudomonas aeruginosa was isolated from the brain, blood and intestine of one animal and from the intestine of four others. No other pathogens were isolated.

Subcutaneous inoculations of infected swine brain suspension into rabbits failed to effect any reaction, thus eliminating the possibility of the presence of Aujeszky's disease virus. The symptoms, course of the disease, mortality rate, and histological examination of the brain excluded the possibility of the presence of Teschen's disease virus. Chicken embryo inoculations were negative.

DISCUSSION

The two main objectives in this investigation were to study the characteristics of a variant strain of virus isolated from pigs dying as the result of a field vaccination failure and to maintain these characteristics over a number of serial passages.

The outstanding characteristics of this virus, referred to as Virus "A," have been its ability to produce a disease with encephalitic symptoms, short incubation period, a short course and severe lesions. It does not necessarily mean that encephalitic symptoms manifested by convulsive seizures identifies this virus as one of the variant types observed during the 1949-50 period of increased vaccination failures. It is, however, perhaps significant that during this period the editor of Veterinay Medicine (1951) commented that "outbreaks of unidentified nervous disorders in swine have been reported by practitioners in widely separated localities in the cornbelt." An excellent description of convulsive activity in hog cholera infected pigs was given by Brunschwiler (1925). The convulsions described were of the same nature as those observed in virus "A" infected animals. The observation of Quin (1950) that convulsions occur in peracute hog cholera cases likewise can be said to be true of virus "A" infected animals. Hutyra et al. (1946)

acknowledge that in hog cholera cases, "exceptionally there occur convulsions and various compulsive movements with marked lethargy." This helps to confirm the associations of convulsions with hog cholera infections but further accentuates the fact that frequent observation of such a phenomenon as in virus "A" infected animals, is unusual. Hofferd's (1948) suggestion of the relationship of dietary deficiency to convulsive seizure was interpreted to mean possibly calcium deficiency. The injection of 25 cc of calcium gluconate intravenously between convulsive seizures did not prevent the recurrence of convulsions several minutes later. Ray (1945) discussed the encephalitic reaction of streptococcic infected swine, but cultures of the brains of virus "A" infected animals revealed no streptococci or other pathogens.

Subcutaneous injections of rabbits with brain suspension of infected swine produced no reaction and eliminated the possibility of Aujeszky's disease. The course and mortality of the infection as well as the examination of histological sections of brain and cord, eliminated Teschen's disease.

The short incubation of virus "A" infections was shown by the rapid body temperature rise and the quick drop of T.W. B.C. A comparison of the rapid rise of temperature in virus

"A" infected pigs with results obtained from the use of other viruses by other investigators showed that virus "A" had a much shorter incubation period than viruses studied by Dorset (1922), Shope (1929) and Köbe (1934). Dorset's work indicated that scarcely more than one-fourth of the animals had a rise of body temperature on the third day. The Federal Bureau of Animal Industry (1949) prohibits the use of swine which are showing visible signs of illness on or before the third day for the production of inoculation virus. Virus "A" infected animals showed 79.5 percent to have high temperatures on the second day and almost 100 percent on the third day. Most of these animals were visibly sick on the third day.

Shope's (1929) virus increased in virulence following serial intraperitoneal passage. At first the regular incubation period was four to seven days. In later passages the incubation period was reduced to three days. In contrast, virus "A" demonstrated a short incubation and rapid course immediately upon field isolation.

The T.W.B.C. of virus "A" infected pigs was not noticeably different from the determinations made on the pigs injected with virulent viruses by Shope (1929) and Kernkamp

(1939). There was, however, a marked difference between the effects of virus "A" on T.W.B.C. and the effects of two commercial viruses of blood origin. Virus "A" caused a far more rapid drop of T.W.B.C. than did the commercial viruses.

The course of the disease was marked by frequent acute deaths. Deaths as early as three days and less rarely at five and six days have occurred. However, some animals have survived as long as 48 days. The mortality has been 100 percent, for all animals have died or become unable to rise and take nourishment and were destroyed. There have been no recoveries in animals not receiving serum.

Dale et al. (1950) tested a variant strain of virus with simultaneous injection of BAI experimental serum No. 1 in swine and found the serum to be ineffective in 15 to 20 cc doses. This serum was also ineffective when used simultaneously with virus "A" in similar doses. While the percent of protection afforded by the serum against the Michigan virus appeared greater than against the variant strain of Dale, it will be observed that lighter pigs (35 to 40 pounds) were used in the Michigan experiment than in the experiment of Dale et al. (1950) in which pigs from 40 through 150 pounds were used.

Commercial serum tested in a like manner proved more effective than the BAI serum but did not completely neutralize the Michigan virus "A" in all cases.

Difference in types of inoculating materials must be considered in all comparative tests. Without exception, the viruses with which virus "A" has been compared have been of blood origin. Without exception, virus "A" has been of brain tissue origin. However, it was used throughout the experiment in a 20 percent suspension in broth or saline. Theoretically this would appear to make it more dilute than virus of blood origin since the virus seems to have an affinity for vascular endothelial tissue.

In the absence of titration of the virus it is not clear as to the fundamental reason for lack of protection afforded by a serum of known potency. Two factors that may be responsible are differences either in antigenicity or virulence of the virus. In view of the lack of sufficient evidence indicating antigenic variation of virus "A" it must be recognized that all of the immunological differences observed could be explained on the basis of increased virulence.

This factor was emphasized by Ruppert (1930), who suggested that great variations exist in the virulence of hog cholera virus. He believed that an immunity brought about by a weak virus could be overcome by a strong virus.

A study of the pathological changes observed in animals infected with virus "A" verified the findings of Seifried (1932) and Bueno (1944), both of whom were convinced that swine fever was a disease of the reticulo-endothelial system. Lesions of the brain and digestive system have further demonstrated the effects of virus "A" on vascular endothelial cells. The hydropic degeneration and proliferation of vascular endothelial cells as well as perivascular cuffing contributed markedly to the constriction and occlusion of the lumen of involved capillaries and arterioles. The constriction of the vessel lumen would slow the blood stream and contribute to the sludging action described by Beamer et al. (1949). The sludging would in turn promote occlusion of tightly constricted vessels, since there seems to be a migration of the leucocytes to the periphery of vessels in these areas (Plate XXI). This migration, typical in the formation of a thrombus, might be the first stage in the occlusion of the vessel. With the occlusion of the vessel it

was relatively easy to conceive of the formation of infarcts described in the stomach and in the large intestine. The development of ulcers in the infarcted area subsequently infected with facultative pathogenic coliform bacteria followed logically as demonstrated in the results of this work.

Gibbs (1933) demonstrated that hog cholera virus could be isolated from button ulcers of runty pigs 42 to 72 days following an attack of hog cholera. Doyle (1946) fed viscera from field cases of enteritis to cholera susceptible and hog cholera immune pigs and obtained enteritis in only the hog cholera susceptible group. These findings help identify necrotic enteritis of the button ulcer type and general type with the disease of hog cholera.

It was also possible to explain the convulsive action exhibited by the swine in this experiment as the direct result of occlusion of blood vessels. Most convulsions occurred when a nervous type of pig was first aroused in the morning in a comparatively early stage of the disease. At this stage the animal was still relatively easily excited, and probably still had strong heart action. When aroused in the morning there was sudden excitement. The heart sent a sudden flow of blood, that most

likely was already sludged, through vessels nearly occluded by an acute attack of the virus on the vascular endothelium. This could have resulted in the packing of cells in the narrowed lumen with the subsequent severe passive hyperemia and consequent intense pain which stimulated a convulsive seizure.

With this idea in mind, we could probably classify virus "A" as an agent with a pronounced affinity for vascular endothelial tissue particularly in the brain and intestines as the result of either a variation in antigenicity, virulence, tissue selectivity, or tissue affinity.

Acute rickets has not previously been recognized as an integral part of the picture presented by an animal infected with hog cholera. It is, however, quite evident that acute rickets does occur and is directly or indirectly due to the virus. Although it was not demonstrated that the blood supply to the costochondral junction was disturbed, a partial occlusion of arterioles could easily have resulted in malnutrition of the area through lowered blood supply. One should couple this idea with the fact that the hog cholera infected pigs lost appetite on the second or third day following inoculation and consumed little or no feed from that time until death. Add to these points

the fact that the very acuteness of the lesions indicate that the duration of rickets has been shorter than the course of the virus infection. One should recall also that an adequate supply of vitamin D (1,200 units per pound of feed) and a sufficient amount of complete mineral mixture (3 percent of total feed) was fed to all animals for at least one week and to some for more than a month prior to the virus inoculation.

In summarizing these items it not only seems possible but quite logical that rickets in a very acute but definite stage should be a part of the pathological picture presented by animals infected with hog cholera.

The somewhat frequent severe medullary hemorrhages seen in the kidneys of virus "A" infected animals, exemplified in a way, the severity of lesions produced by this virus. Dorset (1904) described ecchymotic hemorrhages and clots in the pelvis as being seldom seen in chronic hog cholera but common in acute cases. Seifried and Cain (1932), however, rarely found petechial and ecchymotic hemorrhages in the medulla and pelvis.

The general tendency in the present attack on the variant problem seems to have centered about the removal of the

variant from commercial hog cholera virus products or the prevention of the variant's appearance. This is in direct contrast to the views of Hilleman et al. (1950) on influenza virus. These authors propose that consideration be given to the elimination of older strains of the virus used in vaccine production and the substitution of more recently isolated strains. This suggestion was made to counteract the slow but clearly evident variation of influenza virus over the past few years.

We cannot be certain that the variation observed in the hog cholera virus maintained in this laboratory is antigenically the same as that of the viruses tested by the Bureau of Animal Industry, although we feel that the immunological response to virus "A" follows Dale's et al. (1950) description closely. Neither can we be certain at this point that the virus is antigenically different from the strains of virus widely studied in Europe and in the United States.

If we are to assume, however, that an antigenic difference does exist, one may wonder if there is a justification for attempting to eliminate the variant virus from the immunizing products as the final solution to the vaccination problem. What assurance is there that the origin of this variant virus lies in

the manipulation of this virus within the serum establishment and is thus controllable by careful technic? Is it possible that a field virus might undergo this variation naturally or exist in the field as an antigenically distinct strain and find its way into pigs used in the commercial production of virus?

Since one of the foremost advantages of the use of serum and virus treatment in the control of hog cholera is its reliability in areas of active infection, would one be justified in relying entirely upon increased serum dosage rather than serum specificity as a means of combating field exposure when vaccinating in such areas?

Although these questions have been asked by many, the answers given seemed to indicate a degree of uncertainty.

The development of a reliable means of successfully reproducing the variant virus in serial animal passage seemed to pose a serious problem.

Virus "A" reproduced in the experimentation described in this paper, has maintained characteristics which at this time must be considered as being those of a variant in either virulence or antigenicity. Serial passage of virus "A" has been maintained throughout 16 intracranial swine passages without an increase

in either the incubation period, the course of the disease or without losing its encephalitic tendencies. No simultaneous inoculation with small doses of anti hog cholera serum was needed to retain these characteristics as suggested by Dale et al. (1950.

SUMMARY AND CONCLUSIONS

1. An encephalitic strain of hog cholera virus was isolated and maintained through sixteen intracranial passages in pigs without the loss of ability to produce encephalitic symptoms or other characteristics.

2. Encephalitic symptoms occurred just as frequently in subcutaneously inoculated swine as in intracranially inoculated ones.

3. This virus, designated as virus "A," produced a short incubation period as indicated by increased body temperature of inoculated pigs to 105.0° F. or higher 24 hours to 72 hours following inoculation of the virus.

4. A short incubation period was further indicated by the rapid drop of total white blood cell numbers 24 to 72 hours following inoculation.

5. The course of disease caused by virus "A" was short with deaths occurring as early as three to five days following inoculation.

6. In 100 animals inoculated there were no recoveries. All animals died or were moribund when destroyed.

7. Incomplete protection against virus "A" was provided by Federal Bureau of Animal Industry experimental serum No. 1.

8. Commercial serum did not give complete protection in regulation serum test doses.

9. Pigs immunized with commercial serum and virus were protected against a 2 cc challenge dose of virus "A" but showed some reaction.

10. Efforts to increase the virulence of the virus by use of penicillin in place of filtration for preparation of a bacteria-free inoculum, apparently were without success.

11. The use of vitamin E to increase the susceptibility of the animals to the virus infection appeared to be slightly successful.

12. Thalamic lesions were evident in 100 percent of all cases studied. Severity of lesions appeared greatest in animals dying between the tenth and fourteenth days.

13. A commercial virus tested in pigs produced almost no lesions of the brain.

14. Hydropic degeneration and proliferation of the vascular endothelium of the intestine and perivascular infiltration of vessels of the brain appear to be the primary pathological changes with hemorrhages and other lesions secondary or the result of these changes.

15. Gastric ulcers and colonic button ulcers have been shown to be due to infarction as a result of blood vessel damage.

16. Rickets has been demonstrated in hog cholera infected pigs maintained on adequate rations.

17. No pathogenic bacteria other than Pseudomonas aeruginosa was isolated from the brain, blood or intestine. This organism was isolated from five cases.

BIBLIOGRAPHY

Andrewes, C. H.
A change in rabbit fibroma virus suggesting mutation. I. Experiments on domestic rabbits. J. Exptl. Med., 63: 157-171, 1936.

Atherton, I. K.
Hog cholera control versus prevention. J. Amer. Vet. Med. Assoc., 17: 278-287, 1923-1924.

Baker, James A.
Serial passage of hog cholera virus in rabbits. Proc. Soc. Exptl. Biol. and Med., 63: 183-187, 1946.

Beamer, P. D.; E. H. Bloch, L. Warner, F. Brooks, J. A. Anliker and M. H. Knisely
Sludged blood in three young pigs experimentally infected with hog cholera. Amer. J. Vet. Res., 10: 111-114, 1949

Boynton, William H., Gladys M. Wood, F. W. Wood, and N. H. Casselberry
Cell changes in the gall bladder as an aid in the diagnosis of hog cholera. Proc. Forty-fifth Ann. Meeting U. S. Livestock San. Assoc., 44-47, 1941.

Boynton, William H., William N. Takahoshi, Gladys M. Woods and Wesley W. Walker
Further studies on propagation of hog cholera virus in vitro including electron micrographs. Vet. Med., 43: 403-406, 1948.

Brunschwiler, Karl
Uber Meningitis acuta und verwante Zustande beim Schwein. Zeitschr. Infekionskrankh. Parasitare Krankh. u. Hyg. Haustiere 28: 277-294, 1925.

Bueno, P.
Alteracoes cardiacas encontradas em casos de peste suina. Arquivos do Instituto Biologico Sao Paulo, 11: 59-68, 1949.

Bureau of Animal Industry, United States Department of Agriculture.
Inoculation virus. Code of Federal Regulations, p. 118.8, 1949.

_____.
Variant of hog cholera virus brings losses for a second year. Vet. Med., 45: 457, 1950.

_____.
Minimum dosage. Code of Federal Regulations, CFR Amendment 51-6, p. 119.63, 1951.

Cahill, E. A.
The significance of post vaccination trouble. J. Amer. Vet. Med. Assoc., 64: 171-183, 1923.

_____.
Post vaccination trouble—a possible diagnostic method. J. Amer. Vet. Med. Assoc., 27: 425-431, 1928-1929.

Cole, C. G.
Leucocyte counts on the blood of normal, cholera infected and recently immunized pigs. J. Amer. Vet. Med. Assoc., 34: 392-400, 1932.

Connaway, J. W., and A. J. Durant
Experiments on compliment fixation—hog cholera. Missouri Agr. Exper. Sta. Bul. 131: 486-487, 1915.

Dale, C. N., H. W. Schoening, C. G. Cole, R. R. Henley and M. R. Zinober
Variations (variants) of hog cholera virus. J. Amer. Vet. Med. Assoc., 118: 279-285, 1950.

De Kock, G., E. M. Robinson and J. J. G. Keppel
Swine fever in South Africa. Onderstepoort J. Vet. Sci. and Animal Ind., 14: 31-93, 1940.

De Schweinitz, E. A., and M. Dorset
New facts concerning the etiology of hog cholera. Bureau of Animal Industry, Twentieth Annual Report, 157-162, 1903.

Dinwiddie, R. R.
Studies on the hematology of normal and cholera-infected hogs. Uni. of Ark. Agr. Exper. Sta., Tech. Bul. 120: 41 pp., 1941.

Dorset, M.
Review of research of hog cholera. U. S. Livestock San. Assoc., Proc. of Twentieth Annual Meeting, 42-50, 1916.

_____.
Report of experiments with Suipestifer bacterins. Proc. Twenty-fifth Ann. Meeting, U. S. Livestock San. Assoc., 146, 1921.

_____.
A note on the period of incubation of hog cholera. J. Amer. Vet. Med. Assoc., 61: 393-396, 1922.

Dorset, M., B. M. Bolton and C. N. McBryde
The etiology of hog cholera. U. S. Dept. of Agr., Bureau of Animal Industry, Twenty-first Annual Report, 138-158, 1904.

Dorset, M., C. N. McBryde and W. B. Niles
Further experiments concerned with the production of immunity from hog cholera. U. S. Dept. of Agr., Bureau of Animal Industry, Bul. 102: 96 pp., 1908.

Doyle, L. P., and R. S. Spray
Pathogenic bacteria in hog cholera blood. J. Inf. Dis., 27: 245-249, 1920.

Doyle, L. P., and F. L. Walkey
Attempts to produce enteritis in swine. J. Amer. Vet. Med. Assoc., 109: 280-282, 1946.

Dunne, Howard W.
White blood cell determinations in hog cholera cases complicated with pneumonia. M. S. C. Vet., 8: 127-129, 1948.

Dunne, H. W., R. W. Luecke, W. N. McMillen, M. L. Gray and Frank Thorp, Jr.
The pathology of niacin deficiency in swine. Amer. J. Vet. Res., 10: 351-356, 1949.

Editor's note
Vet. Med. 46: 177, 1951.

Findlay, G. M.
New strains of animal viruses. Royal Society of London, Proceedings Series B, 125: 303-305, 1938.

Galloway, I. A.
Discussion on the routes of infection and the paths of transmission of viruses. Proc. of the Royal Society of Medicine, 29(part 1): 570-575, 1936.

Gibbs, Charles S.
Filterable virus carriers. J. Inf. Dis., 53: 169-174, 1933.

Goldner, Jacques
A modification of the Masson trichrome technique for routine laboratory purposes. Amer. J. Path., 14: 237-243, 1938.

Graham, R.
Botulism in swine and its relation to immunization against hog cholera. J. Amer. Vet. Med., 60: 76-79, 1921.

Hammon, W. McD.
Arthropod-born virus encephalitidies. Amer. J. Trop. Med., 28: 515-525, 1948.

Haring, C. M.
The control of hog cholera. J. Amer. Vet. Med. Assoc., 48: 239-247, 1916.

Healy, Daniel J., and Wallace V. Smith
Complement fixation in hog cholera. J. Inf. Dis., 17: 213-218, 1915.

Hecke, Friedrich
Die kunstliche Vermehrung des Scheinepestvirus mittels Gewebekulturen. Zentralbl. Bakt. I Abt. Orig. 126: 517-526, 1932.

Helmbolt, C. F., and E. L. Jungherr
The neuropathologic diagnosis of hog cholera. Amer. J. Vet. Res., 11: 41-49, 1950.

Hewitt, E. A.
Certain chemical and morphologic phases of the blood of normal and cholera-infected swine. I. S. C. J. Sci., 4: 143-249, 1932.

Hilleman, M. R., R. P. Mason and N. G. Rogers
Laboratory studies on the 1950 outbreak of influenza. Pub. Health Rep., 65: 771-794, 1950.

Himmelberger, L. R.
Cell inclusions in hog cholera. J. Amer. Vet. Med. Assoc., 48: 450-453, 1916.

Hofferd, R. M.
Diagnosis and control of some swine diseases. Cornell Vet., 34: 152-173, 1944.

Hoskins, H. Preston
Some observations on hog cholera and the use of serum. Amer. Vet. Rev., 47: 46-56, 1915.

Hupbauer, A.
Zur Frage der Pluralitat des Schveinepestvirus. Zeitschr. Infektionskrankh., Parasitare Krankh. u. Hyg. Haustiere 45: 294-300, 1934.

Hutyra, Franz, Joseph Marek and Rudolph Manninger
Special pathology and therapeutics of the diseases of domestic animals. Alexander Eger, Chicago, Ed. 5: 286-312, 1946.

Jacatot, H.
Sur le domaine zoologique du virus de la peste porcine. Bull. Acad. Vét. France, 10: 282-283, 1937.

Johnson, Eunice, F. N. Andrews and C. L. Shrewsbury
The preparation of muscular tissue for histological study. J. Animal Sci., 2: 244-250, 1943.

Kaplan, M. M., and D. R. Meranze
Porcine virus encephalomyelites and its possible biological relationship to human poliomyelitis. Vet. Med., 43: 330-341, 1948.

Kernkamp, H. C. H.
The blood picture in hog cholera. J. Amer. Vet. Med. Assoc., 95: 525-529, 1939.

Kernkamp, H. C. H., and M. H. Roepke
The invitro neutralization of hog cholera virus with hog cholera antiserum. Amer. J. Vet. Res., 3: 300-303, 1942.

Kernkamp, H. C. H., and R. Fenstermacher
Subtypical hog cholera. Proc. Fifty-first Ann. Meeting U. S. Livestock San. Assoc., 96-99, 1947.

King, Walter E., and Robert H. Wilson
Studies on hog cholera and preventive treatment. Kansas State Agr. Col. Exp. Sta. Bul. 171: 139-195, 1910.

Kinsley, A. T.
A century of hog cholera. J. Amer. Vet. Med. Assoc., 83: 449-463, 1933.

Köbe, K., and W. Schmidt
Differentialdiagnose zwischen chronischer schweinepest und ferkelgrippe. Deutsche Tierärztl. Wchnschr. 42: 145-148, 1934.

Koprowski, Hilary, Terence R. James and Herald R. Cox
Propagation of hog cholera virus in rabbits. Proc. Soc. Exptl. Biol. and Med., 63: 183-187, 1946.

Lammert, H. W., Jr., and H. Moussatche
Adaptation of yellow fever virus to young chickens by serial brain to brain passage. J. Inf. Dis., 72: 228-231, 1943.

Lewis, P. A., and R. E. Shope
The blood in hog cholera. J. Exptl. Med., 50: 719-736, 1929.

Lillie, R. D.
Histopathologic technic, p. 36-37, 1948. The Country Life Press, Garden City, N. Y.

Montgomery, R. Eustace
On a form of swine fever occurring in British East Africa (Kenya Colony). J. Comp. Path. and Therap., 34: 159-191, 1921.

Moore, V. A.
History and distribution of hog cholera in America. J. Amer. Vet. Med. Assoc., 48: 208-212, 1916.

Muir, R. O.
A search for diagnostic symptoms and lesions in a variety of small experimental animals after administration of swine fever tissue suspensions by different routes. J. Comp. Path. and Therapeutics, 53: 237-243, 1943.

Munce, T. W., and H. A. Hoffman
Vaccination shock in young pigs (anti-cholera serum shock). N. Amer. Vet., 11: 37-49, 1930.

Neiman, I. S., and Oram C. Woolpert
Intracerebral inoculation of fetal guinea pigs with bacille Calmette-Guerin and the H37 strain of tubercle bacillus. Amer. J. Path., 12: 153-164, 1936.

Petrunkevitch, Alexander
New vixing fluids for general purposes. Sci., 77: 117-118, 1933.

Quin, A. H.
The past and future of hog cholera control. J. Amer. Vet. Med. Assoc., 116: 411-415, 1950.

Ray, J. D.
Enteritis and mixed infections in swine. Vet. Med., 40: 48-54, 1945.

_____.
Current hog cholera problems. Vet. Med., 43: 236-237, 1948.

_____.
Infectious diseases of swine. Vet. Med., 45: 15, 1950.

Reichel, John
Fixed hog cholera virus. J. Inf. Dis., 12: 106-110, 1913.

Reynolds, M. H.
Hog cholera serum work with special references to disappointments. Proc. Amer. Vet. Med. Assoc., p. 519-527, 1912.

Rohrer, H., and A. Graf
Die Bedeutung des "selbstandigen encephalitischen Symptomenkomplexes" fur die histologische Diagnose der chronischen Schweinepest. Deutsche tierarzl. Wchnschr. 42: 637-643, 1934.

Ruppert, Fritz
Uber Virulenzschwankungen des Virus der Schweinepest. Miessner-Festschrift, Fritz Eberlein, Hannover, 167-173, 1930.

Salmon, D. E.
Hog cholera: its history, nature and treatment as determined by the inquiries and investigations of the Bureau of Animal Industry. Gov. Print. Office, Washington, 197 pp., 1889.

Sarnowiec, W.
Au sujet de la nouvelle méthode de diagnostic de la peste du porc. Bull. Acad. Vét. France, 7: 201-204, 1934.

Scott, Joseph P.
Swine influenza associated with hog cholera. An abstract. J. Bact., 45: 303, 1943.

Siefried, O., and C. B. Cain
Histological studies on hog cholera. I. Lesions in the central nervous system. J. Exptl. Med., 53: 277-288, 1931.

_____.
Histological studies on hog cholera. II. Lesions of the vascular system. J. Exptl. Med., 56: 345-351, 1932.

_____.
Histological studies on hog cholera. III. Lesions in the various organs. J. Exptl. Med., 56: 351-362, 1932.

Sippel, W. L.
The Boynton gall bladder smear for diagnosing hog cholera. Cornell Vet., 35: 147-151, 1945.

Anonymous
Agricultural Statistics. U. S. Dept. of Agr., U. S. Government Printing Office, p. 362, 1949.

Taylor, R. M.
Studies on survival of influenza virus between epidemic and antigenic variants of the virus. Amer. J. Pub. Health 39: 171-178, 1949.

Tenbroeck, Carl
Cultivation of the hog cholera virus. J. Exptl. Med., 74: 427-432, 1941.

Theiler, M.
Studies on the action of yellow fever in mice. Annals of Trop. Med. and Parasit., 24: 249-272, 1930.

Thorp, Frank Jr., and Robert Graham
A study of the leucocyte changes in the blood of diseased swine. J. Amer. Vet. Med. Assoc., 30: 198-203, 1930.

Van Es, L., and J. F. Olney
Collected observations pertaining to hog cholera. Uni. Nebr. College of Agri., Agri. Exper. Sta. Res. Bul. 135: 35 pp., 1944.

Wright, John T., and Karl Habel
A comparison of antigenicity and certain biological characteristics of six substrains of Pasteur fixed rabies virus. J. of Immunology, 60: 503-515, 1948.

Young, George A.
Influenza in swine. Vet. Med., 45: 141-146, 1950.

_____.
Personal communication. Nov. 27, 1950.

Zichis, J.
Studies on hog cholera virus. J. Amer. Vet. Med. Assoc., 272-277, 1939.

APPENDIX

TABLE VIII

SUMMARY OF THE GROSS LESIONS OF 84 EXPERIMENTALLY INFECTED HOG CHOLERA PIGS

Lesion	Mild	Moderate	Severe	Total	% of Total
Conjunctivitis	10	24	25	59	70.2
Erythema	11	9	5	25	29.8
Subcutaneous hemorrhage	-	1	1	2	2.4
Lymphatic peripheral hemorrhage	17	15	8	40	47.6
Lymphatic diffuse hemorrhage	12	33	15	60	71.4
Tonsillitis	11	13	8	32	38.1
Epiglotis - petechiation	13	5	2	20	23.8
Hydrothorax	5	9	0	14	16.8
Hemothorax	2	3	0	5	6.0
Hydroperitoneum	2	6	1	9	10.7
Thymus - petechiation	-	-	1	1	1.2
Hydropericardium	6	3	1	10	11.9
Epicardial petechiation	2	5	4	11	13.1
Myocardial degeneration	19	9	2	30	35.7
Coronary occlusion	1	2	0	3	3.6

TABLE VIII (Continued)

Lesion	Mild	Mod-erate	Se-vere	Total	% of Total
Fibrinous pericarditis	0	3	1	4	4.8
Pleuritis	0	2	0	2	2.8
Bronchopneumonia	30	12	7	49	58.3
Pneumonic ecchymosis	1	5	7	13	15.7
Interstitial edema	0	0	1	1	1.9
Atelectasis	3	2	0	5	6.0
Peritonitis	1	2	0	3	3.6
Gastritis	16	13	9	38	45.2
Gastric edema	1	1	0	2	2.4
Gastric petechiation-serosa	0	2	1	3	3.6
Gastric petechiation-mucosa	1	4	2	7	8.3
Enteritis - small intestine	9	3	1	13	15.5
S. intest. serosal and mucosal petechiation	0	1	0	1	1.2
Colitis, cecitis, diffuse necrotic	6	5	3	14	16.8
Acute colic ulceration	0	0	1	1	1.2
Button ulcers	7	5	5	17	20.2

TABLE VIII (Continued)

Lesion	Mild	Mod-erate	Se-vere	Total	% of Total
Colonic petechiation	1	4	4	9	10.7
Nutritional enteritis	3	0	0	3	3.6
Acute catarrhal colitis	9	7	1	18	21.4
Hemorrhagic colitis	1	-	2	3	3.6
Splenic infarction	5	13	5	23	27.4
Renal cloudy swelling	9	3	2	14	16.8
Renal petechiation cortex	25	19	10	54	64.3
Renal ecchymosis-cortex	3	0	10	13	15.5
Renal ecchymosis-pyramids	8	7	9	24	28.6
Nephritis	0	2	0	2	2.4
Hepatic fatty metamorphosis	0	6	0	6	7.1
Cholecystic congestion	2	1	0	3	3.6
Cholecystic petechiation	3	3	3	9	10.7
Cholecystic echymosis	2	2	1	5	6.0
Hepatic scars	2	14	4	20	23.8

TABLE VIII (Continued)

Lesion	Mild	Mod-erate	Se-vere	Total	% of Total
Ascariasis	2	6	3	11	13.1
Cystic petechiation	40	22	4	66	78.6
Cerebral congestion	15	31	15	61	72.6
Acute rickets	27	12	2	41	46.4
Petechiation of omentum and messentery	0	0	1	1	1.2

TABLE IX

SIMULTANEOUS SERUM AND VIRUS IMMUNITY EXPERIMENTS USING COMMERCIAL SERUM AND VIRUS "A"

Pig No.	Wt. in lbs.	Virus		Inoculation Route	Serum Amt.	Highest Temp.	T.W.B.C.		Convulsions	Survival Period
		No.	Amt.*				Norm.	Low		
130	80	5-1C	1 cc	I C	5 cc	106.6	16,000	5,700	None	6 days
131	80	5-1C	1 cc	I C	5 cc	107.1	18,250	5,300	Severe	8 days
126	60	4-1C	1 cc	S	15 cc	107.4	17,900	5,100	None	17 days
123	60	4-1C	0.5cc	I C	15 cc	104.4	18,000	9,600	None	No reaction
167	35	9-1C	2 cc	S	15 cc	106.0	8,850	8,300	None	Mild illness
168	35	9-1C	2 cc	S	15 cc	103.8	18,300	7,100	None	No reaction
169	35	9-1C	2 cc	S	15 cc	105.8	14,200	7,100	None	Mild illness

TABLE IX (Continued)

Pig No.	Wt. in lbs.	Virus		Inoculation Route	Serum Amt.	Highest Temp.	T.W.B.C.		Convulsions	Survival Period
		No.	Amt.*				Norm.	Low		
170	35	9-1C	2 cc	S	15 cc	105.2	13,950	6,500	None	Mild illness
127	60	4-1C	1 cc	S	30 cc	105.0	19,300	5,800	None	Sick - recovered
124	60	4-1C	0.5cc	I C	30 cc	105.0	18,200	6,550	None	Sick - recovered

* Amount of 20% Brain Suspension
I C - Intracranial
S - Subcutaneous

TABLE X

SIMULTANEOUS SERUM AND VIRUS IMMUNITY EXPERIMENTS USING BAI EXPERIMENTAL SERUM NO. 1 AND VIRUS "A"

Pig No.	Wt. in lbs.	Virus		Inoculation Route	Serum Amt.	Highest Temp.	T.W.B.C.		Convulsions	Survival Period
		No.	Amt.*				Norm.	Low		
140	35	6-IC	1 cc	I C	10 cc	107.0	16,700	7,800	None	18 days
141	35	6-IC	1 cc	I C	10 cc	105.2	15,250	5,200	None	Sick - recovered
142	35	6-IC	1 cc	I C	10 cc	105.2	14,600	6,800	None	Sick - recovered
174	40	9-IC	2 cc	S	15 cc	103.8	27,750	9,800	Severe	5 days
175	40	9-IC	2 cc	S	15 cc	105.8	22,500	3,900	None	14 days
176	40	9-IC	2 cc	S	15 cc	106.3	12,800	5,100	None	19 days
177	40	9-IC	2 cc	S	15 cc	104.4	14,450	8,200	None	Mild illness

TABLE X (Continued)

Pig No.	Wt. in lbs.	Virus		Inoculation Route	Serum Amt.	Highest Temp.	T. W. B. C.		Convulsions	Survival Period
		No.	Amt.*				Norm.	Low		
204	35	11-IC	2 cc	S	15 cc	105.0	12,650	11,300	None	27 days
205	35	11-IC	2 cc	S	15 cc	105.2	12,850	4,000	None	Sick - recovered
206	35	11-IC	2 cc	S	15 cc	105.4	13,250	8,550	None	Sick - recovered
207	35	11-IC	2 cc	S	30 cc	104.0	13,700	8,900	None	No reaction
208	35	11-IC	2 cc	S	30 cc	105.0	13,100	8,950	None	Mild illness
209	35	11-IC	2 cc	S	30 cc	103.8	22,150	11,800	None	No reaction

* - Amount of 20% brain suspension. I C - Intracranial. S - Subcutaneous.

TABLE XI

SIMULTANEOUS SERUM AND VIRUS AND IMMUNITY EXPERIMENTS

Pig No.	Virus		Inoculation Route	Vaccinated or Recovered	T.W.B.C.		Reactions	Survival Period
	No.	Amt.*			Norm.	Low		
4	"A"	0	Contact	Vaccinated**	19,550	8,550	Diarrhea	Sick - recovered
107	2-1C	0.5 cc	S	Vaccinated**	21,900	15,400	-	No reaction
108	2-1C	0.5 cc	S	Vaccinated**	24,100	10,400	Diarrhea	Sick - recovered
109	2-1C	0.5 cc	S	Vaccinated**	20,350	8,950	Diarrhea	Sick - recovered
106	2-1C	3 cc	S	Recovered***	19,800	16,400	No reaction	No reaction

* Amount of 20% Brain Suspension.

** Vaccinated two months prior to this experiment. Commercial serum and virus were used.

1C - Intracranial

S - Subcutaneous

*** Recovered Pig No. 4, two months after contact.

TABLE XII

THE EFFECT OF AUREOMYCIN ON THE COURSE OF VIRUS "A" IN SIX ARTIFICIALLY INFECTED SWINE

Days After Inoc.	No Aureomycin		Aureomycin 10 gm. per 100 lbs. Feed*	
	Av. Temp.	Average W.B.C. (3 pigs)	Av. Temp.	Average W.B.C. (3 pigs)
1	103.3	-	102.3	-
2	104.6	-	106.1	-
3	106.5	-	106.5	-
4	107.2	8,733	106.9	7,117
5	106.9	10,917	106.3	7,217
6	106.9	7,783	108.2	6,817
7	107.3	6,417	107.0	4,333
8	105.6	6,100	105.7	4,933
9	106.1	6,233	105.4	6,333
10	103.5	7,325	104.6	5,566
	Deaths occurred on 12th and 15th days (one animal was killed accidently on 9th day by anterior venacava bleeding)		Deaths occurred on 15th, 21st and 18th days. The latter animal suffered convulsions on the 11th day.	

* Animal No. 465 which provided the virus for the succeeding intracranial passages was in this group.

TABLE XIII

SERIAL INTRACRANIAL PASSAGE OF VIRUS "A" IN SWINE

Days After Inoc.	Passage No. 1, Virus 465						Passage No. 2, Virus 101			
	101		102		103		104		105	
	Temp.	W. B.C.	Temp.	W. B.C.	Temp.	W. B.C.	Temp.	W. B.C.	Temp.	W. B.C.
N		17,000	-	18,750	-	17,500	104.2	21,350	103.8	16,200
1	104.0	-	104.8	-	104.6	-	105.2	15,300	106.4	10,100
2	106.8	-	105.0	-	106.4	-	106.6	9,800	105.8	4,500
3	106.2	7,900	106.6	10,950	106.4	8,950	106.8	5,150	105.6	4,250
4	106.2	6,550	106.2	6,200	105.2	5,850	Died 4th day		104.2	4,700
5	106.2	3,700	106.8	4,700	104.0	4,900			106.2	-
6	103.6	-	107.4	-	107.2	-			106.2	3,250
7	103.8	7,900	106.2	7,300	106.0	8,200			106.4	-

TABLE XIII (Continued)

Days After Inoc.	Passage No. 1, Virus 465						Passage No. 2, Virus 101			
	101		102		103		104		105	
	Temp.	W. B.C.	Temp.	W. B.C.	Temp.	W. B.C.	Temp.	W. B.C.	Temp.	W. B.C.
8	Died 8th day		106.2	6,700	104.6	6,500			105.8	4,100
9			105.0	7,550	105.2	6,250			105.0	-
10			105.6	7,750	105.8	9,450			106.0	4,600
11			105.8	5,250	105.0	-			104.0	6,600
12			106.2	6,700	104.4	6,950			105.2	6,750
13			105.4	8,850	104.6	5,950			105.8	6,600
14			104.0	-	104.0	-			101.0	-
15			104.4	5,600	104.4	10,000			Died 15th day	

TABLE XIII (Continued)

Days After Inoc.	Passage No. 1, Virus 465						Passage No. 2, Virus 101			
	101		102		103		104		105	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
16			104.4	9,650	103.1	19,700				
17			103.0	9,150	103.6	-				
18			Destroyed 18th day		102.8	20,150				
19					102.4	-				
20					102.6	26,100				
21					Died 21st day					

Underlined numbers indicate convulsions on that day.

TABLE XIV

SERIAL PASSAGE OF "A" VIRUS

Days After Inoc.	Intracranial Passage No. 3, Virus No. 104					
	113		114		115	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	102.6	20,200	103.0	21,600	102.2	22,450
1	103.8	-	103.0	-	103.4	-
2	105.0	-	104.8	-	105.8	-
3	105.8	7,300	105.6	10,100	105.6	-
4	106.2	3,650	106.0	4,700	105.8	4,350
5	105.6	6,750	105.4	6,800	104.7	6,250
6	106.0	8,100	106.2	8,250	106.0	11,800
7	107.2	———	106.0	-		Died 6th day
8	105.6	5,550	105.4	7,500		
	Destroyed 21st day		Destroyed 48th day			

TABLE XIV (Continued)

Days After Inoc.	Subcutaneously Inoculated Controls					
	110		111		112	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	102.4	18,700	102.6	20,250	102.6	23,250
1	104.4	-	103.6	-	103.4	-
2	105.2	-	105.4	-	105.2	-
3	106.6	6,800	105.4	8,900	106.0	9,900
4	106.8	5,900	105.6	9,400	105.8	7,600
5	104.8	10,700	105.6	19,250	105.4	6,600
6	105.4	8,200	106.2	20,000	104.6	16,200
7	103.6	_____	106.0	_____	105.1	_____
8	106.2	9,400	104.8	11,350	102.0	16,650
	Died 8th day		Died 11th day		Died 11th day	

_____Underlined numbers indicate convulsions on that day.

TABLE XV

SERIAL PASSAGE OF "A" VIRUS

Days After Inoc.	Intracranial Passage No. 4, Virus No. 115					
	116		117		118	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	104.2	16,050	103.8	14,600	104.2	15,750
1	104.1	-	104.0	-	104.4	-
2	104.8	8,650	106.8	5,100	106.4	3,650
3	105.6	-	106.0	-	106.4	-
4	104.9	8,900	105.0	9,200	106.4	7,200
5	106.2	-	105.2	-	105.2	-
6	104.8	9,800	Died 6th day		104.6	7,100
7	-	-			Died 6th day	
8	-	11,150				

TABLE XV (Continued)

Days After Inoc.	Subcutaneously Inoculated Controls					
	119		120		121	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	104.6	13,900	103.6	14,250	103.8	13,800
1	104.0	-	104.4	-	104.2	-
2	105.8	7,400	106.4	9,300	106.0	6,200
3	106.6	-	106.5	-	106.6	-
4	107.0	10,150	106.0	12,050	107.0	11,300
5	106.2	-	108.6	-	108.2	-
6	104.2	-	107.6	10,250	107.4	11,450
7	Died 7th day		-	-	-	-
8			-	6,200	-	6,250
			Destroyed 14th day		Destroyed 11th day	

Underlined numbers indicate convulsions.

TABLE XVI

INTRACRANIAL SERIAL PASSAGE OF "A" VIRUS

Days After Inoc.	Passage No. 5, Virus No. 117		Passage No. 6, Virus No. 122			
	122		128		129	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	104.0	17,850	103.6	19,150	102.2	17,200
1	105.0	-	-	-	-	-
2	103.6	-	107.4	6,600	107.0	7,800
3	105.6	7,300	106.0	6,500	104.5	6,450
4	105.4	7,650	106.0	9,850	104.0	11,000
5	106.6	7,100	-	-	-	-
6	107.2	10,100	103.5	5,450	102.6	4,750
7	106.4	8,300	Destroyed 6th day		104.6	-
8	105.0	Died 8th day			104.0	6,800
					Died 25th day	

TABLE XVI (Continued)

Days After Inoc.	Passage No. 7, Virus No. 128					
	137		138		139	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	-	16,250	-	15,300	-	14,850
1	105.0	-	105.2	-	105.4	-
2	104.8	11,100	104.4	8,900	104.4	10,100
3	105.4	10,100	105.4	8,750	102.8	10,050
4	106.4	6,700	106.0	10,900	106.6	8,700
5	-	-	-	-	-	-
6	107.0	4,300	107.0	5,200	107.2	5,350
7	105.6	-	107.4	-	106.8	-
8	104.0	10,200	105.8	11,100	106.4	10,050
	Destroyed 23rd day		Died 21st day		Destroyed 36th day	

Underlined numbers indicate convulsions.

TABLE XVII

SERIAL INTRACRANIAL PASSAGE OF "A" VIRUS

Days After Inoc.	Passage No. 8, Virus No. 128			
	147		148	
	Temp.	W.B.C.	Temp.	W.B.C.
N	103.2	16,950	103.4	15,800
3	104.2	-	106.0	-
5	105.8	-	107.4	-
7	106.6	21,000	107.6	6,300
8	-	-	-	-
10	106.8	22,350		
	Destroyed 10th day		Destroyed 14th day	

Days After Inoc.	Subcutaneously Inoculated Control			
	149		150	
N	103.8	17,100	103.2	16,850
3	107.6	-	106.0	-
5	107.4	-	107.0	-
7	107.8	9,700	106.6	9,100
8	106.8	5,600		
10	Died 10th day		Died 13th day	

Underlined numbers indicate convulsion on that day.

TABLE XVIII

SERIAL INTRACRANIAL PASSAGE OF "A" VIRUS

Days After Inoc.	Passage No. 9, Virus No. 147					
	159		160		161	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	104.0	17,250	104.1	15,100	103.4	18,600
3	106.0	-	100.8	-	106.0	-
4	106.4	8,750	99.6	6,450	104.0	11,050
5	107.6	-	Destroyed 4th day		105.0	Severe
6	105.8	4,250			104.0	18,000
7	102.0	7,500			105.0	-
11	Destroyed 7th day				105.4	9,575
14					100.0	8,800
					Destroyed 14th day	

TABLE XVIII (Continued)

Days After Inoc.	Subcutaneously Inoculated Control					
	162		163		164	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	103.6	11,300	103.8	15,750	103.6	18,100
3	106.8	-	104.8	-	106.2	-
4	105.6	5,950	105.2	12,950	106.4	8,750
5	106.6	-	105.4	-	107.6	Very Severe
6	106.4	3,500	105.9	6,500	100.8	44,000
7	Died 6th day		105.0	-	Died 6th day	
11			105.0	4,350		
			Died 11th day			

Underlined numbers indicate convulsions on that day.

TABLE XIX

SERIAL INTRACRANIAL PASSAGE

Days After Inoc.	Passage No. 10, Virus 159 and 160				Subcutaneous Inoc. Control			
	186		187		178		179	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	104.4	15,750	104.2	16,300	104.2	19,650	104.6	28,350
1	-	-	-	-	-	-	-	-
2	104.4	-	104.8	-	102.6	8,700	103.0	8,300
3	105.5	-	106.5	-	106.4	8,900	105.6	8,600
4	104.6	25,150	103.0	25,750	106.7	4,700	106.4	4,200
5	106.6	8,650	105.0	45,800	106.0	5,150	106.4	4,800
6	106.0	4,600	106.0	6,200	106.6	8,350	106.6	5,300
7	105.2	4,600	105.4	4,300	106.8	16,400	106.7	30,100

TABLE XIX (Continued)

Days After Inoc.	Passage No. 10, Virus 159 and 160				Subcutaneous Inoc. Control			
	186		187		178		179	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
8	102.6	4,100	105.0	5,500	106.2	5,550	Destroyed 7th day	
9	105.0	4,250	104.2	6,100	103.8	22,050*		
10	Destroyed 9th day		104.8	7,000	104.6	15,700*		
11			104.8	6,200	105.6	20,450*		
12			Destroyed 11th day		105.8	22,150*		
13					104.8	19,100*		
14					105.0	132,000*		
15					99.4	86,200*		
					Destroyed 15th day			

* Severe nervous symptoms.

TABLE XX

SERIAL INTRACRANIAL PASSAGE

Days After Inoc.	Passage No. 11, Virus 186 and 187							
	188		189		190		191	
	Temp.	W.B.C.	Temp.	W.B.C.	Temp.	W.B.C.	Temp.	W.B.C.
N	102.4	21,200	103.4	20,350	103.0	18,700	103.6	16,200
1	-	-	-	-	-	-	-	-
2	103.6	13,000	104.0	8,100	105.0	7,600	103.0	5,600
3	104.8	3,000	105.8	2,850	106.0	8,700	104.8	5,600
4	105.0	8,550	105.0	13,000	106.0	8,700	102.8	7,900
5	105.6	6,600	105.4	7,200	106.0	7,900	105.6	5,900
6	106.8	8,700	105.2	5,700	106.0	17,100	104.2	13,500
7	104.0	15,000	104.6	3,450	104.8	45,600	105.2	12,900
8	-	-	-	-	Dead 8th day		-	-
9	104.4	22,400	104.4	17,600			103.2	45,700
10	-	9,700	Destroyed 9th day				Destroyed 9th day	
	Destroyed 10th day							

TABLE XXI

SERIAL INTRACRANIAL PASSAGE COMPARISON BETWEEN PENICILLIN TREATED AND FILTERED VIRUS

Days After Inoc.	Passage No. 12, Virus No. 188, 189, 190 and 191, With Penicillin					
	198		199		200	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	105.2	11,900	104.2	12,250	103.4	11,800
1	-	-	-	-	-	-
2	103.4	9,600	102.8	12,100	104.0	7,400
3	106.6	10,000	107.8	10,100	104.4	5,700
4	107.2	5,350	106.4	13,300	102.0	40,800
5	106.6	7,200	107.4	8,600	Destroyed 4th day	
6	105.4	12,100	105.2	5,600		
7	105.6	4,850	106.0	16,400		
	Died 17th day		Died 15th day			

TABLE XXI (Continued)

Days After Inoc.	Passage No. 12, Virus No. 188, 189, 190 and 191 Filtered					
	201		202		203	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	105.2	12,700	104.0	12,300	104.2	11,950
1	-	-	-	-	-	-
2	104.0	7,900	101.6	10,300	102.8	10,250
3	107.0	7,300	106.2	7,600	105.2	8,400
4	106.0	6,950	106.4	6,250	104.6	4,500
5	106.8	12,200	107.2	6,900	104.8	4,200
6	105.9	17,000	105.0	6,900	105.0	4,500
7	104.2	50,000	104.4	14,800	103.8	19,700
8	Destroyed 7th day		Destroyed 10th day		Died 15th day	

_____ Indicate convulsions occurring on that day.

TABLE XXII

SERIAL INTRACRANIAL PASSAGE

Days After Inoc.	Passage No. 13, Virus No. 200 and 201, Plus 5 cc Vit. E Subcut.					
	218		219		220	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	102.8	17,900	102.8	19,250	102.0	16,000
1	104.8	-	105.0	-	105.0	-
2	105.6	7,950	105.0	25,400	104.4	6,300
3	106.4	5,250	105.4	12,500	106.4	5,600
4	107.4	3,500	104.8	11,400	106.6	3,250
5	107.4	2,850	105.6	6,250	104.6	3,200
6	105.2	5,300	105.4	6,500	106.6	3,400
7	104.6	4,600	105.0	8,200	107.0	3,100
8	105.8	-	104.2	-	102.0	Convul.
	Destroyed 13th day		Died 13th day		Destroyed 9th day	

TABLE XXII (Continued)

Days After Inoc.	Passage No. 13, Virus No. 200 and 201, No Vit. E					
	221		222		223	
	Temp.	W. B. C.	Temp.	W. B. C.	Temp.	W. B. C.
N	102.0	19,150	102.8	16,800	103.6	18,650
1	105.2	-	104.0	-	103.2	-
2	105.0	9,300	104.4	18,000	104.0	10,300
3	105.8	11,500	106.0	7,500	105.8	6,300
4	104.4	6,600	106.0	6,600	106.6	6,200
5	104.2	6,350	104.6	13,850	105.8	6,000
6	105.0	13,100	104.4	4,400	105.2	5,450
7	103.4	15,200	104.0	3,400	103.0	3,600
8	103.8	-	104.0	-	105.0	-
	Convulsions 11th day					
	Destroyed 11th day		Destroyed 14th day		Destroyed 13th day	

TABLE XXIII

CHALLENGE OF A RECOVERY PIG AND PREVIOUSLY IMMUNIZED PIGS

Days After Inoc. (1)	Recovery Pig*		3 Month Vaccinated Pig**					
	106		107		108		109	
	Temp.	W.B.C.	Temp.	W.B.C.	Temp.	W.B.C.	Temp.	W.B.C.
N	102.4	19,850	102.8	21,900	103.8	24,100	103.4	20,350
1	104.4	-	103.0	-	104.0	-	103.2	-
2	103.8	-	103.2	-	103.4	-	103.4	-
3	104.0	16,400	103.8	19,700	103.7	14,500	104.4	8,950
4	103.4	18,750	104.8	15,400	104.4	10,400	104.6	10,350
5	102.8	20,100	103.6	20,500	103.4	15,750	104.0	14,900
6	104.0	19,100	103.4	18,000	103.6	13,900	103.4	11,300
7	103.4	-	101.6	-	102.0	-	100.0	-
8	103.0	20,100	103.8	19,200	103.4	11,600	103.0	22,250

* No. 106 was vaccinated by serum and virus simultaneous method, and later contact exposed to virus "A" and recovered.

** Simultaneous commercial serum and virus vaccinated three months prior to this experiment.

(1) Dose of virus for No. 106 was 3 cc, for the others, 0.5 cc.

TABLE XXIV

IMMUNITY EXPERIMENTS WITH SWINE* RECEIVING 15 cc OF BAI EXPERIMENTAL SERUM NO. 1 AND 2 cc VIRUS "A" 9th PASSAGE

Days After Inoc.	174	175	176	177	178	179	180
	15 cc Serum	15 cc Serum	15 cc Serum	15 cc Serum	No Serum	No Serum	Con-tact
N	27,750	22,500	12,800	14,450	19,650	28,350	18,600
2	10,100	22,000	9,100	11,400	8,700	8,300	18,300
3	10,000	20,100	9,000	10,100	8,900	8,600	18,450
4	9,800	12,000	7,600	8,200	4,700	4,200	10,100
5	10,200	10,000	7,750	9,100	5,150	4,800	10,250
6	Died 5th day	12,400	6,900	10,600	8,350	5,300	9,850
7		12,350	14,100	11,050	16,400	30,100	19,800
8		14,300	5,150	25,850	5,550	Destroyed 7th day	18,700
9		4,800	15,900	31,400	22,050		15,700

TABLE XXIV (Continued)

Days After Inoc.	174	175	176	177	178	179	180
	15 cc Serum	15 cc Serum	15 cc Serum	15 cc Serum	No Serum	No Serum	Con-tact
10		4,600	5,500	29,900	15,700		12,100
11		24,650	7,000	28,900	20,450		21,150
12		6,900	6,800	24,300	22,150		24,100
13		7,100	5,100	21,100	19,100		10,000
14		3,900	10,000	67,000	132,000		5,700
15		Destroyed 15th day	-	Recovered	86,200		-
19			21,250		Destroyed 15th day		19,700
			Destroyed 19th day				Destroyed 19th day

* Swine averaged 40 pounds in weight.

_____Underlined numbers indicate convulsions occurring on that day.

TABLE XXV

THE EFFECT OF ULTRACENTRIFUGATION OF HOG CHOLERA VIRUS WITH REFERENCE TO INCUBATION PERIOD AND COURSE OF THE DISEASE

Days After Inoc.	Com. Virus I 2 cc Subcut.		First Pass. Virus No. 182 Ultracent. Subcut. 1 cc	
	182		211	
	Temp.	W. B. C.	Temp.	W. B. C.
N	104.2	15,850	103.8	17,100
1	-	-	103.4	22,600
2	104.4	32,000	105.0	5,900
3	104.0	15,900	104.4	7,000
4	105.6	15,600	105.6	5,600
5	105.0	8,400	106.2	3,350
6	106.4	9,150	105.6	4,600
7	105.6	6,700	105.8	8,100
8	105.6	4,100	104.8	-
9	105.0	6,750	105.0	26,200
10	104.0	8,300	103.8	10,800
11	Died 10th day		Destroyed 10th day	

TABLE XXV (Continued)

Days After Inoc.	Commercial Phenolized Virus II 2 cc Subcut.					
	68	78	79	90	92	95
N	24,150	19,250	26,100	44,400	24,250	34,050
1	25,200	16,900	23,250	16,700	17,200	20,600
2	23,250	10,600	12,500	10,300	30,500	18,400
3	11,000	17,900	15,700	8,350	7,950	4,900
4	17,500	7,850	14,900	7,150	11,475	10,150
5	18,850	7,150	11,400	5,950	12,400	8,030
6	9,850	5,350	11,250	6,780	7,550	7,250

All animals bled for virus production on 6th day.