A VIRUS-INDUCED EPIZOOTIC HEMORRHAGIC DISEASE OF THE VIRGINIA WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS)*

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PLATES 7 TO 10

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Early in August, 1955, a highly fatal disease of wild deer appeared in New Jersey. It reached epizootic proportions before it finally subsided about mid-September. The region involved included Morris, western Essex, and northern Somerset counties, and was roughly rectangular in shape and approximately 150 square miles in area. The number of deer dying during the outbreak has been estimated at between 500 and 700 animals and 233 carcasses were actually found in the fields and woods of the involved area. Many of the carcasses were in or beside bodies of water when discovered, suggesting that the animals were either thirsty or febrile or both at the time of death.

The disease struck with such suddenness and killed so many deer so rapidly that for a time, in the beginning, there was a suspicion that the condition might be an intoxication perhaps resulting from the ingestion of spray materials or poisonous plants. However, exhaustive toxicological analyses of the gastric contents and of the viscera of dead deer in the laboratories of the New Jersey State Police failed to reveal the presence of any known poison.

In like manner, some aspects of the epidemiology of the ailment suggested that it might be anthrax, pasteurellosis, or even rinderpest. The possibility that the condition was anthrax was ruled out by studies conducted by Dr. Oscar Sussman of the Bureau of Veterinary Public Health, New Jersey State Department of Health. Pasteurellosis was eliminated from further consideration early in the outbreak by work carried out by Dr. R. A. Hendershott of the Division of Animal Industry, New Jersey Department of Agriculture. The disease did not prove to be rinderpest as will be made evident when our experimental findings are described.

As the outbreak progressed and as the disease was observed for a longer period of time, it became more and more apparent that it resembled an in-

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fection—one with which none of us had had previous experience—certainly a disease new to New Jersey and perhaps an entirely new disease. There was no concurrent unusual illness or death loss among either the domestic animals or the other wild animals of the area. The ailment, whatever it was, seemed to be specific for deer. It failed to reoccur in New Jersey in 1956, 1957, 1958, or 1959, and what its subsequent behavior in this State will be remains to be seen.

Picture Presented by the Naturally Occurring Disease.—Most of the deer succumbing during the outbreak died without being seen during the time they were ill. The few that were observed shortly preceding death gave the impression of being in profound shock. Their coats were rough and they lay listlessly on the ground with their necks extended before them and their ears drooping. They had apparently lost their alertness to the approach of man. They could, however, rise to their feet, though they walked with an unsteady wobbly gait. None were observed to be paralyzed nor to have convulsions. Some exhibited a bloody diarrhea. Animals once found when ill seldom lived for more than a few hours.

A more extensive description of the disease, derived from the observation of experimentally infected deer throughout the whole course of their illnesses, will be given later. The pathological picture also will be described later and the description will be based upon the findings observed in the large number of experimentally infected animals that have come to autopsy as well as on the relatively small number of naturally infected deer that were opened and examined cursorily in the field. Although the field autopsies conducted on animals dead for various periods of time during the warm summer weather when the disease prevailed were far from ideal in revealing the true postmortem picture of the disease, enough natural cases were examined soon after death to assure us that the pathological pictures we observed later in our experimental cases faithfully reproduced those which occurred in the natural disease.

Historical.—In the beginning we believed that the condition observed in the New Jersey deer was a new illness and, in a preliminary publication (1) named it "epizootic hemorrhagic disease" (EHD) from its salient clinical and pathological features. Subsequently we found that we had been in error in our belief. Search of the files of the United States Forest Service and of the records of the United States Fish and Wildlife Service by Leonard E. Foote of the Wild Life Management Institute (2) has revealed that outbreaks of a fatal epizootic disease similar to the one we encountered in New Jersey have occurred in deer in various of the southeastern states at irregular intervals at least since 1890. These outbreaks have been variously diagnosed as blackleg, mycotic stomatitis, or hemorrhagic septicemia by the individuals recording them. Among woodsmen and hunters in the South, the disease has long borne the name "black tongue," presumably because one of the characteristics of a deer dead of it was a discolored and swollen tongue which generally protruded from the mouth. Ruff (3) has given a good description of "black tongue" and comparison of his description with our own observations makes it seem most likely that our New Jersey disease was

indeed an outbreak of "black tongue." However, because this name has confusing connotations in connection with established but unrelated diseases in other species, it seems inadvisable to perpetuate it in the scientific literature, and we propose, therefore, to continue to designate the condition as epizootic hemorrhagic disease of deer (EHD).

In addition to the epizootics recorded from the southeastern states, two occurred in the State of Washington in 1946 and 1953. It was during one of these that the first evidence of transmissibility of an infectious entity from deer to deer was obtained when Spencer (4) showed that blood from a sick deer reproduced the disease on inoculation into a normal deer.

In 1955, at about the same time that the outbreak of EHD was occurring in New Jersey, a similar disease appeared in deer in Michigan. This was studied by Fay, Boyce, and Youatt (5) and their observations, like our own preliminary ones (1), indicated that the causative agent of the disease was a filterable virus.

In September and October, 1956, a fatal ailment resembling EHD appeared extensively among deer in South Dakota and Lester M. Berner of the South Dakota Department of Game, Fish, and Parks furnished us with frozen tissues from a fatal case. It is the purpose of the present paper to describe our findings in an experimental study of EHD and to compare the causative agents prevailing in the New Jersey and South Dakota outbreaks.

Materials and Methods

Infectious Material—New Jersey Strain.—Our starting material was obtained from a deer typically ill of the disease at the time it was sacrificed.[1] A suspension of spleen, liver, kidneys, and blood of this animal was employed to infect our first experimental deer. During the first 2 years of work with the disease, we used as infectious inocula mixtures of spleen, liver, and kidneys from experimentally infected deer, ground with sterile sand in a mortar, and suspended to 5 or 10 per cent in either 0.85 per cent salt solution or buffered saline of pH 7.0. Subsequently the spleen has been found to be more regularly infective than either the liver or kidneys, and broth as superior to saline as a suspending medium. Because of this finding, we now employ, as infectious inocula, spleen from experimentally infected deer, ground with sterile sand in a mortar, and suspended to 5 or 10 per cent in beef heart infusion broth of pH 7.4. The inocula are effective in inducing disease when given either subcutaneously or intramuscularly. Organs from infected deer retain their infectivity for long periods of time, as will be indicated more fully later, when kept frozen in sealed containers in a solid CO_2 storage box.

South Dakota Strain.—Our starting material consisted of spleen, liver, and kidneys from a fatal case sent to us frozen in solid CO_2 by Lester M. Berner of the South Dakota Department of Game, Fish, and Parks. The South Dakota agent produced a disease in experimental deer that was similar but sometimes less severe than that caused by the New Jersey agent. It was less stable in saline than the New Jersey agent and suspension in broth was essential to assure its retention of full pathogenicity. The South Dakota agent was stored and passaged in the same manner as the New Jersey agent, and the two were eventually compared for identity, as will be outlined later.

Handling of Experimental Deer.—Most of our experimental deer were obtained in nature as fawns and were bottle-fed on cow's milk until weaning time. After this they were maintained

[1] We are indebted to Mrs. Geraldine R. Dodge for making this deer available to us and to Dr. C. R. Robinson for performing the autopsy on this particular animal.

on a diet of hay and a grain mixture known as omolene (Ralston Purina Co., St. Louis). They have been used when they were between 10 weeks and 2 years of age and age has not been noted to influence their susceptibility to EHD. A few deer obtained by trapping in the wild have ranged in age from 6 months to 5 years, as determined by examination of their teeth. After capture these have been maintained in the same manner as those reared from fawnhood.

Test deer, prior to use, have been kept in covered pens, 7 feet high, and either 6 or 9 feet wide, and 9 feet deep. The pens have a sturdy heavy wooden framework and are lined with 14-gauge welded wire of 2 by 1 inch mesh. Most of our pens are rendered relatively insect-proof by a covering of 20-mesh plastic screen. Depending upon the size of the deer, from two to as many as four animals may be maintained in such pens until ready for experimental use. Pens identical with those described for storage are used for animals under experiment except that here the animals are penned individually. Each pen has a cement floor and is deeply bedded with straw or hay when in use.

Serial Transmission of EHD to Normal Deer.—A 10 per cent suspension in 0.85 per cent NaCl solution of spleen, liver, kidneys, and blood from a New Jersey deer ill of the naturally occurring disease was administered subcutaneously to our first experimental animal, deer 1-5. This fawn developed an illness, typical of the naturally occurring disease, on the 5th day and died on the 6th day after inoculation. Autopsy revealed a picture like that seen in the natural disease. Because we had at this stage of our investigation no knowledge as to the character of the causative agent and no information as to how it might best be preserved for study, it was decided to maintain the disease, if possible, by deer to deer passage until we knew more about the storing qualities and nature of the causative agent.

The virus was passed serially in deer for five passages, as shown in Table I, using 4 ml. of the supernatant of a 10 per cent suspension in saline of spleen, liver, and kidney of each passage animal to administer subcutaneously to the deer of each succeeding passage in all except the third passage. In this passage the supernatant of a 10 per cent suspension in saline of spleen, liver, and kidney of deer 1-6, the second passage animal, was filtered through a sintered glass filter for coarse clarification and then two portions of this glass filtrate were further filtered through separate demonstrably bacteria-tight Berkefeld N filters. The causative agent was present in all three filtrates, as evidenced by the ability of each filtrate, in the usual 4 ml. dose, to infect and kill experimental deer. Two of these animals, deer 1-8 and 1-9, though exhibiting the usual incubation period of 7 days, died less rapidly than was usual. One, deer 1-8, lived for 4 days after first being noted ill, while the other, deer 1-9, underwent a lingering illness of 2 weeks before it finally succumbed. Both showed characteristic gross pathology at autopsy. Similar long periods of survival have subsequently been observed not infrequently in animals inoculated with unfiltered material making it seem unlikely that filtration of the causative agent modified its pathogenicity for deer or removed an etiologically essential component.

By the time our fifth passage deer had succumbed we were in possession of evidence which indicated that the causative agent could be stored frozen or in 50 per cent glycerol-saline for at least as long as 30 days and, because of this, we discontinued our maintenance of the disease by serial passage and resorted to the use of agent stored frozen or in glycerol. Subsequent work, as outlined

in a later section of this paper, indicated that the causative agent of EHD was stable for long periods of time when stored at the temperature of solid CO_2 with or without 50 per cent glycerol-saline.

Disease Picture Presented by Experimentally Infected Deer.—As has been mentioned earlier, only a few naturally infected deer were observed in the wild and these were all very acutely ill, lived only a few hours after being first seen, and presented little opportunity for clinical observation. The 66 deer that have been experimentally infected with the New Jersey strain and the 30 with the South Dakota strain of EHD have given us an opportunity to observe

TABLE I

Serial Passage of the Agent of Epizootic Hemorrhagic Disease in Deer

Passage No.	Deer No.	Inoculum given subcutaneously	Results	
			Incubation period	Died
			days	*day*
	NC-1 (natural case)		Un-known	Moribund when sacrificed
First	1-5	Blood, liver, spleen, and kidneys of deer NC-1	5	6
Second	1-6	Organs* of deer 1-5	7	7
Third	1-7	Berkefeld N filtrate of organs of deer 1-6	7	7
"	1-8	Berkefeld N filtrate of organs of deer 1-6	7	11
"	1-9	Sintered glass filtrate of organs of deer 1-6	7	21
Fourth	2-1	Organs of deer 1-7	7	7
Fifth	2-3	Organs of deer 2-1	7	Moribund 9th day—sacrificed

* Liver, spleen, and kidneys.

the disease throughout its course. The incubation periods have varied from 4 to 12 days, with most animals showing manifest signs of illness on either the 6th or 7th day after inoculation. About half of our experimental deer (37 with the New Jersey strain, and 10 with the South Dakota strain) have run the acute course seen in nature and have died in apparent shock within 24 to 48 hours after having first been noted ill. The most prominent sign in these animals has been prostration of an extreme type. The first signs of illness have been loss of appetite, roughening of the coats, marked injection of the conjunctiva and the mucosa of the lips, and sometimes rather pronounced salivation. Most of our deer have been obstinately averse to permitting the taking of rectal temperature prior to the time they were moribund. The few animals tame enough to be handled have shown a temperature elevation to fever level (40°C. or over) on the last day of their incubation periods and during the early hours of their observable illness. Following this their temperatures have dropped precipitously to become subnormal

just before death (34 to 37°C.). Some of the acutely ill animals have exhibited a terminal bloody diarrhea or epistaxis or both.

Nine experimental deer have been either completely resistant to infection with the New Jersey strain of EHD agent or have been so mildly affected that their illness was not recognized, and four have reacted similarly to the South Dakota agent. Six deer inoculated with the New Jersey strain, and 12 of those infected with the South Dakota agent, have become ill following infection but have recovered after periods of illness of variable duration and severity. Fourteen deer infected with the New Jersey agent and four of those infected with the South Dakota agent have undergone fatal infections in which the period of illness has been longer than the usual acute course seen in the few naturally infected animals we had observed. These deer, though severely ill, lived for periods ranging from 2 days to 2 weeks before succumbing and during this time exhibited signs similar to those seen in the animals that died more rapidly.

Pathology of EHD.—The findings encountered at autopsy in experimental deer dying from infection with either the New Jersey or the South Dakota strain of EHD were both constant and striking and were similar to those seen in the few naturally infected animals that we had the opportunity to examine. In the usual, and what we consider typical, case the following changes were to be observed:

As one reflected the skin from the carcass, petechial and ecchymotic hemorrhages were to be seen in the subcutaneous tissues. These were most marked in the groin, in the axillae, and over the sternum. There was also frequently a gelatinous edema of the subcutaneous tissues, especially in the axillae, over the abdomen, and in the groin. The peritoneal cavity usually contained a moderate to large excess of clear straw-colored or slightly blood-tinged fluid, and the serosal surfaces of the stomachs and intestines were splotched and flecked with myriads of purplish red petechial and ecchymotic hemorrhages. There was sometimes dark blood in the lumen of the small intestine and shed blood sometimes separated the muscle layers of the intestinal walls. The cecum and the first half to two-thirds of the colon frequently contained tarry feces. The contents of the stomachs were ordinarily fluid in character, sometimes bloody, and had a fetid odor. The liver was dark and congested and sometimes showed areas of discrete hemorrhage within its parenchyma. The spleen was not enlarged, though its substance was dark and congested. The kidneys, even in animals autopsied soon after death, were pulpy and friable and the medullary portion was dark purplish red, obviously from extensive hemorrhage and congestion. The lymph nodes in the intestinal mesentery were sometimes dark and diffusely engorged. The pericardial sack ordinarily contained a large excess of clear, straw-colored fluid. The heart itself was speckled with myriads of small purplish red hemorrhages, most marked over the auricles and at the apex of the ventricles. The aorta, pulmonary arteries, and the vena cavae were a splotched purple instead of their normal white color. The lungs were sometimes congested and edematous, though frank pneumonia was not observed. The mucosae of both the esophagus and the trachea were speckled with myriads of petechiae. While no determinations of blood clotting time have been conducted, it has been evident, when autopsying freshly dead deer or those sacrificed when moribund, that the blood remained fluid for an unusually long time after it was shed. The impression gained from postmortem examinations of dead deer was that the animals had bled into their own tissues—a situation which perhaps accounted for the condition of extreme shock which preceded death.

Histopathology of EHD.—The microscopic findings in the tissues and organs of deer dead of EHD were those to have been predicted by the gross changes observed at autopsy.

Accumulations of extravasated red blood cells were present in the parenchyma of the spleen and lymph nodes and in the medullary portion of the kidneys. There were accumulations of red blood cells in the muscle layers of the stomachs and of the gut walls at all levels, in their mucosae, and beneath their serosal linings. The heart musculature, as well as the walls of the large blood vessels within the thorax, contained irregular infiltrating masses of extravasated red blood cells (Fig. 1) and the serosal lining of the heart was separated from the underlying musculature in places by masses of red blood cells (Fig. 2). There were hemorrhages among the cells of the adrenals and of the thymus (Fig. 3) and accumulations of red cells, both in the alveolar walls and in the alveolar spaces of the lung, as well as in the peribronchial tissues. In the kidneys of some of the animals, there were focal areas of tubular necrosis (Fig. 4), perhaps resulting from pressure by accumulations of extravasated blood, and vacuolization of cells of the collecting tubules (Fig. 5). In most tissues, the blood vessels were dilated and packed with red blood cells. This engorgement of distended blood vessels was particularly apparent in sections of the kidneys, small intestine, and trachea (Fig. 6).

Search for microscopic evidence of vascular lesions or changes in blood vessel walls to account for the extensive extravasations of red blood cells found in the various tissues and organs has revealed no constant alterations or imperfections in the vascular bed. The failure to account for the extensive hemorrhages on the basis of microscopically evident anatomical defects in blood vessel walls leaves unexplained the pathogenesis of the hemorrhages encountered in EHD and suggests that in this disease perhaps physiological pathology is of more lethal importance than is anatomical pathology.

Stability of the Agent of EHD in the Frozen State.—Pieces of liver, spleen, and kidney from infected deer, cut into chunks approximately 1 cm. square, have been placed in sterile, screw-capped bottles, either with or without 50 per cent glycerol-saline, and stored in a freeze storage box containing solid CO_2. At varying intervals such tissues have been removed from the freezer and thawed before grinding with sand in a mortar to make 5 or 10 per cent tissue suspensions in saline or broth. Such suspensions prepared from tissues stored in frozen glycerol-saline for periods of 43, 54, 100, and 116 days, have proven lethally pathogenic for experimental deer. Tissues stored in this manner for longer than 116 days have not been tested for infectivity because by this time we had evidence to indicate that storage frozen, without the presence of glycerol, was effective. We have on numerous occasions produced characteristically lethal disease in experimental deer with suspensions prepared from tissues stored frozen in the CO_2 box for periods of 4 months or longer, and in three instances, the only times tested, tissues stored for 9 months proved lethally pathogenic. While longer periods of storage have not been tested, it is apparent that the causative agent of EHD is stable when stored at low temperature.

Bacteriology of EHD.—No single type of bacterium either aerobic or anaerobic, has been found to be regularly present in the spleens, livers, kidneys, and heart bloods of deer dead or dying of EHD. Very often suspensions of these organs have proven bacteriologically sterile, though still capable of inducing disease in experimental deer. In addition, bacteriologically sterile Berkefeld filtrates of suspensions of organs from infected deer have proven capable of inducing characteristic and fatal disease in

experimental deer. These findings, showing that bacterial pathogens do not play an etiological role, when considered with those discussed earlier concerning the filterability of the causative agent and its stability in glycerol or in the frozen state, indicate that the causative agent of EHD of deer is a filterable virus. As will be described in the next section, this virus, in the few deer in which it fails to cause a fatal infection, induces the formation of specific virus-neutralizing antibodies.

Neutralizing Antibodies for the Virus of EHD in the Blood Serum of Recovered Deer.—Sera of deer recovered from infection with either the New Jersey or the South Dakota strains of virus have been tested for their capacity to neutralize the homologous strain of virus. The New Jersey convalescent serum was derived from three animals, deer 1-4, 2-5, and 2-7, and was employed either pooled or individually, while the South Dakota serum was from a single recovered animal, deer 8-6.

The virus neutralization tests were conducted in the following manner:

One ml. of the supernatants of 10 per cent suspensions in saline or broth of liver, spleen, and kidney, or of spleen alone, from infected animals, were mixed with 3 ml. amounts of convalescent serum. Control mixtures contained the same amount of virus suspension mixed with 3 ml. of either saline, broth, or normal deer serum. The 4 ml. mixtures were administered to experimental deer, either subcutaneously or intramuscularly, without preceding incubation, but usually after storage frozen at the temperature of solid CO_2 for 24 hours. The results obtained with sera tested against the homologous strains of virus are recorded in Table II.

As shown in Table II, the sera of deer convalescent from infection with the New Jersey strain of EHD neutralized a fatal dose of the homologous virus in seven out of the eight neutralization tests conducted. In test 3, the convalescent serum failed to protect deer 6-5, and the animal, though living longer than its control, succumbed eventually. In both tests with South Dakota virus, serum of a deer convalescent from that strain of virus neutralized it. The findings recorded demonstrate that specific virus-neutralizing antibodies are present in the sera of deer that have recovered from EHD.

Distribution of New Jersey Strain EHD Virus in Infected Deer.—When we began our work with EHD, we used suspensions prepared from liver, spleen, and kidney tissue of infected deer as a source of virus, assuming that all three of these organs probably contained the virus. This assumption proved to be not always correct, as we found when we tested these organs individually for virus. The results obtained are outlined in Table III.

As shown in Table III, virus was present in the spleens of all three of the infected deer studied but was not detectable in the liver or kidneys in two of the three tests made. Since making these observations, we have altered our routine for the preparation of infectious suspensions and now employ spleen alone. Similar studies of the distribution of South Dakota strain virus have not been conducted but with this strain also the spleen has proven to be a constant source of virus.

Attempts to Infect Animals Other Than Deer with the EHD Virus.—Tissue suspensions containing EHD virus, demonstrably lethally pathogenic for deer, have failed to cause observable illness when administered by various routes to rabbits, guinea pigs, hamsters, puppies, swine, adult and new born mice, sheep, or calves. Further-

more the virus failed to grow either in embryonating hens' eggs inoculated by various routes[2] or in tissue cultures of deer kidney cells.[3]

TABLE II

Neutralization of the Virus of EHD with Sera from Recovered Deer

Test No.	Deer No.	Neurtralization test		Results
		Virus strain	Mixed with	
1	3-5	New Jersey	Saline	Killed 9th day when moribund
	3-6	" "	New Jersey convalescent deer serum	No illness
2	5-1	New Jersey	Saline	Died—22 days
	4-8	" "	New Jersey convalescent deer serum	No illness
3	6-4	New Jersey	Saline	Died—12 days
	6-5	" "	New Jersey convalescent deer serum	" 20 "
4	7-2	New Jersey	Saline	Died—11 "
	7-3	" "	New Jersey convalescent deer serum	No illness
5	7-5	New Jersey	Normal deer serum	Died—10 days
	7-3A	" "	New Jersey convalescent deer serum	No illness
6	7-3B	New Jersey	Normal deer serum	Died—10 days
	7-7	" "	New Jersey convalescent deer serum	No illness
7	8-4	New Jersey	Normal deer serum	Died—9 days
	8-5	" "	New Jersey convalescent deer serum	No illness
8	1-09	New Jersey	Normal deer serum	Died—9 days
	1-11	" "	New Jersey convalescent deer serum	No illness
9	9-5	South Dakota	Normal deer serum	Died—10 days
	9-4	" "	South Dakota convalescent deer serum	No illness
10	1-05	South Dakota	Normal deer serum	Ill 7 days and recovered
	1-04	" "	South Dakota convalescent deer serum	No illness

Lack of Relationship of EHD Virus to the Agent of Epidemic Hemorrhagic Fever of Man or the Equine Arteritis Virus.—Certain of the clinical and pathological features

[2] We are indebted to Miss Elizabeth B. Jackson for making some of the embryonated egg cultures.

[3] We are indebted to Dr. Donna Chaproniere for conducting the work with the deer kidney cell cultures.

of EHD suggested a possible similarity to Korean epidemic hemorrhagic fever of man or to the disease caused in horses by the equine arteritis virus. To test whether the resemblance in either case was more than a superficial one, material from these two diseases was administered to experimental deer.

Deer 2-2 was injected subcutaneously with whole blood obtained at the height of illness from one case of epidemic hemorrhagic fever and with a suspension of liver, spleen, kidney, tunic, and testes gotten at postmortem from a fatal case of the disease.[4] The deer remained completely normal throughout a 3 week period of observation. At the end of this time it was challenged subcutaneously with New Jersey strain EHD virus. It sickened typically and died on the 8th day of characteristic EHD.

TABLE III

Distribution of the New Jersey Strain of EHD Virus in the Organs of Infected Deer

Organ from infected deer No.	Organ tested	Deer inoculated No.	Results
7-5	Spleen, liver, kidney	6-8	Died—10 days
"	" " "	8-0	" —17 "
"	Kidney	8-1	No illness
"	Liver	8-2	" "
"	Spleen	8-3	Died—7 days
8-4	Kidney	8-8	" —13 "
"	Liver	8-9	No illness
"	Spleen	9-0	Died—10 days
1-12	Kidney	1-22	No illness
"	Liver	1-21	Died—11 days
"	Spleen	1-23	" —11 "

It was apparent from this result that inoculation with material presumably containing the causative agent of Korean epidemic hemorrhagic fever had not only failed to induce illness in a deer but furthermore had not modified the subsequent susceptibility of this animal to EHD virus.

Two animals, deer 3-3 and 3-4, were inoculated subcutaneously with a suspension of equine spleen and lung containing the Bucyrus strain of equine arteritis virus.[5] Neither animal sickened and both later proved fully susceptible to challenge with New Jersey strain EHD virus and died typically of their challenge infections. Furthermore, EHD virus injected into the fetuses of two pregnant mares yielded negative results.

These findings indicated that EHD virus and equine arteritis virus are not the same agent.

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[4] We are grateful to Dr. Joseph E. Smadel for supplying us with the epidemic hemorrhagic fever material.

[5] We are indebted to Dr. E. R. Doll for furnishing us the Bucyrus strain of equine arteritis virus and for testing the pathogenicity of EHD virus in the fetuses of two pregnant mares.

Attempts to Learn Mode of Transmission of EHD.—We have, on numerous occasions, placed normal deer in the same pens with infected animals and have permitted them to remain in contact until the death of the infected deer. In no case has the exposed animal developed either illness or immunity as a result of this experience. In two instances we have exposed deer by pen contact to a series of fatal cases without the transmission of disease. One of these animals was exposed consecutively twice and the other one three times. They ate from the same feed boxes and drank from the same buckets as their infected pen mates.

It is apparent from these findings that whatever the natural mode of transmission of the virus of EHD may be it is not by contact.

We have had one accidental infection in a penned deer that occurred under circumstances suggesting that the virus may have been transmitted to it by biting stable flies (*Stomoxys calcitrans*). This animal was in an unscreened pen in the neighborhood of two other unscreened pens containing infected deer. The animal was observed to have been badly bitten by *Stomoxys* the week before its illness and death. However, flies collected from the pen of this animal, ground and suspended in saline, failed to cause illness when injected into a susceptible experimental deer.

Subsequent attempts to transmit EHD virus by means of the fly, *S. calcitrans*, or two species of mosquitoes, *Culex pipiens*, and *Aedes vexans*,[6] have failed. Transmission was attempted not only in natural feeding experiments but by the inoculation of susceptible deer with ground suspensions of the insects that had fed on infected deer.

Although all of our insect transmission experiments have thus far yielded negative results, none have been perfect enough in their execution to be certain that technical difficulties inherent in conducting such experiments with animals as obstrepterous as deer have not contributed to our failures.

An Immunological Difference between the New Jersey and South Dakota Strains of EHD Virus.—Deer that have recovered from infection with either the New Jersey or the South Dakota strain of EHD virus are immune to reinfection with the homologous strain. Furthermore the sera of such recovered animals, as shown in Table II, neutralize the homologous virus. However, although the signs of illness and postmortem pictures exhibited by deer infected with the two strains of virus are similar, except for the lesser lethality of that caused by the South Dakota virus, it has been found that the two strains differ from one another immunologically. This difference, sometimes demonstrable by cross-infection tests, is better shown by cross-neutralization tests.

Five deer that were refractory to or recovered from infection with New Jersey strain virus and ten deer that were refractory to or recovered from infection with the South Dakota strain virus were challenged by inoculation with heterologous strain virus. The results obtained are recorded in Table IV.

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[6] We are indebted to Dr. Elton J. Hansens and Dr. Paul P. Barbutis for furnishing us with laboratory-reared flies and mosquitoes and for giving us advice and assistance in the conduct of our insect transmission experiments.

As shown in Table IV, two of the ten deer that had undergone non-fatal infections with the South Dakota strain of EHD virus were still susceptible to infection with the New Jersey strain and underwent characteristic illnesses. However, all five of the deer that had previous infections with New Jersey virus resisted subsequent infection with the South Dakota strain.

This finding indicated that although infection with New Jersey strain virus appeared solidly to protect deer against subsequent infection with South

TABLE IV

Protection Conferred by the New Jersey and South Dakota Strains of EHD Virus against Infection with the Heterologous Agent

Deer No.	Infecting strain of virus	Challenge strain of virus	Results
5-3	South Dakota	New Jersey	No illness
5-7	" "	" "	Moribund 5 days—killed
6-6	" "	" "	No illness
8-5	" "	" "	" "
9-1	" "	" "	" "
9-2	" "	" "	Died—13 days
1-05	" "	" "	No illness
1-06	" "	" "	" "
1-07	" "	" "	" "
1-08	" "	" "	" "
5-5	Control—not infected	" "	Died—8 days
9-0	" " "	" "	" —10 "
1-09	" " "	" "	" —9 "
3-6	New Jersey	South Dakota	No illness
4-0	" "	" "	" "
4-1	" "	" "	" "
4-6	" "	" "	" "
8-2	" "	" "	" "
5-3	Control—not infected	" "	Sick and recovered
9-2	" " "	" "	" " "

Dakota virus, the protection in the reverse direction was less perfect. It was apparent that the two strains of virus were immunologically related.

To obtain further information concerning the immunological relationship between the two strains of virus, cross-neutralization experiments were conducted. These were performed, as described earlier, using the same convalescent deer sera that had previously (Table II) been shown to be effective in neutralizing the homologous viruses. The results are recorded in Table V.

The findings recorded in Table V are clear cut in demonstrating that the serum of deer convalescent from infection with either strain of virus, though

TABLE V

Attempted Cross-Neutralization of the New Jersey and South Dakota Strains of EHD Virus with Sera from Recovered Deer

Test No.	Deer No.	Neutralization test		Results
		Virus strain	Mixed with	
1	5-7	South Dakota	Saline	Sick and recovered
	5-8	" "	New Jersey convalescent deer serum	Died—8 days
2	6-6	" "	Saline	Sick and recovered
	6-7	" "	New Jersey convalescent deer serum	Died—20 days
3	7-0	" "	Saline	Sick and recovered
	7-1	" "	New Jersey convalescent deer serum	Died—10 days
4	8-6	" "	Normal deer serum	Sick and recovered
	8-7	" "	New Jersey convalescent deer serum	Died—11 days
5	9-5	" "	Normal deer serum	Died—10 days
	9-4	" "	South Dakota convalescent deer serum	No illness
	9-3	" "	New Jersey " " "	Sick and recovered
6	1-05	" "	Normal deer serum	Sick and recovered
	1-04	" "	South Dakota convalescent deer serum	No illness
	1-06	" "	New Jersey " " "	Sick and recovered
	1-07	" "	" " " " "	Sick and recovered
	1-08	" "	" " " " "	Sick and recovered
7	9-6	New Jersey	Saline	Died—10 days
	9-7	" "	South Dakota convalescent deer serum	" —14 "
8	1-09	" "	Normal deer serum	Died—9 "
	1-11	" "	New Jersey convalescent deer serum	No illness
	1-10	" "	South Dakota " " "	Died—10 days
9	9-8	" "	Normal deer serum	Died—10 "
	1-19	" "	New Jersey convalescent deer serum	No illness
	1-18	" "	South Dakota " " "	Died—9 days

capable of neutralizing the homologous virus, does not neutralize the heterologous strain.

In the first four neutralization tests with South Dakota virus, in which the control deer sickened and survived while those receiving New Jersey strain convalescent serum died, the suggestion was apparent that something in the New Jersey convalescent serum had enhanced the pathogenicity of South Dakota strain virus. This observation was not repeated, however, in the four deer that received New Jersey convalescent serum in tests 5 and 6 and it therefore seems most likely that the apparent enhancement of pathogenicity of South Dakota virus by New Jersey antiserum in the first four tests must be attributed to the chance selection in those four tests of pairs of deer of differing native susceptibility to the lethal effects of South Dakota virus. At any rate, whatever the correct explanation of the differences may be, there is no doubt that the sera of deer convalescent from infection with New Jersey strain EHD virus failed to neutralize South Dakota virus. In like manner, the serum of a deer convalescent from infection with South Dakota virus failed to neutralize New Jersey strain EHD virus.

It is evident from the findings that the New Jersey and South Dakota EHD viruses, though related, as shown by cross-immunity tests, are not immunologically identical with one another.

DISCUSSION

Epizootic hemorrhagic disease of deer is of interest from three standpoints. One of these is epidemiological and concerns its mysteriously explosive appearance in a population of deer inhabiting a relatively small geographical area, and its later almost as sudden disappearance from that area. The second is immunological and concerns the occurrence of the causative virus in two serological types. The third has to do with the pathological manifestations of the disease and the suggestion, from the findings, that the lethal pathology may be more the result of physiological than anatomical defects.

The disease appeared suddenly in a circumscribed region of New Jersey and in a relatively brief period of time killed an estimated 500 to 700 deer. We have no notion how or from where the virus came to cause this first recognized outbreak in the area. Also we have no information to explain why it suddenly stopped and has failed to reappear in the four succeeding years. That it did not cease because of exhaustion of the supply of susceptible deer is attested by the fact that two deer, trapped in October 1955 from a herd of about 40 animals in which the known death loss only 2 months earlier had exceeded 50 per cent, were still fully susceptible to experimental infection.[7] Although the mode of transmission of the causative virus is not known, our findings indicate that it is not by simple case-to-case contact. Obviously much remains to be learned about the natural history of the disease.

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[7] We are grateful to Mrs. Geraldine R. Dodge for making these animals available to us from the portion of the deer herd on her estate that survived the epizootic.

A second feature of EHD that makes it unusual, its causation by a virus of more than one serological type, is one shared by certain other virus diseases such as foot-and-mouth disease, vesicular stomatitis, and influenza. Although the New Jersey and the South Dakota strains of virus cause disease that is similar, except for the somewhat lesser lethality of that caused by the South Dakota agent, the two viruses are serologically different from each other. The serological differences between the viruses and the similarity of the disease picture that each causes in deer are such as to justify their classification as two immunological types of the same virus. It will be of interest, as viruses from other outbreaks of the disease are studied, to learn whether the New Jersey and South Dakota strains represent the only serological types in which the virus prevails or whether perhaps still other serological types may exist.

A third remarkable characteristic of EHD, and one considered only superficially in our investigation, concerns the pathological manifestations of the disease. Hemorrhages in the form of petechiae, ecchymoses, or gross extravasations of blood into the solid viscera, into the lumen and walls of the gastrointestinal tract, and beneath all serosal surfaces constitute the major pathological changes to be observed, and probably account for the salient signs, those of profound shock, observed in affected animals just prior to death. However, despite all this evidence of extravascular blood, histological examination has failed to reveal the presence of anatomical changes in the vascular bed to account for it. It would appear possible that some basic defect in the blood-clotting mechanism, evidenced by the unusually long periods of time that blood from deer with EHD remains fluid, might account for the pathological findings in the disease and that the salient pathology might, therefore, have a physiological rather than an anatomical basis.

SUMMARY

A circumscribed natural outbreak of a highly fatal disease of deer, which we have designated epizootic hemorrhagic disease (EHD), has been studied. The disease has proven readily transmissible in deer but not in other experimental or domestic animals tested, nor in embryonating eggs or deer kidney cell cultures. The causative agent is a virus which is readily filterable and is capable of storage, either frozen or in glycerol, for relatively long periods of time. It produces a solid immunity in the few animals that survive and the blood sera of such convalescent animals contain virus-neutralizing antibodies. The disease is one in which large and small hemorrhages occur in both the viscera and skeletal structures of the body, as well as in the subcutaneous tissues. It is probably the same as one known popularly in the southeastern United States as "black tongue" of deer. It is unrelated to epidemic hemorrhagic fever of man or to the disease caused in horses by the equine arteritis virus. At least two serologically different types of EHD virus exist. The New Jersey strain is of greater lethality for experimental deer than the serologically

different one obtained from an outbreak that occurred in South Dakota a year after the New Jersey epizootic.

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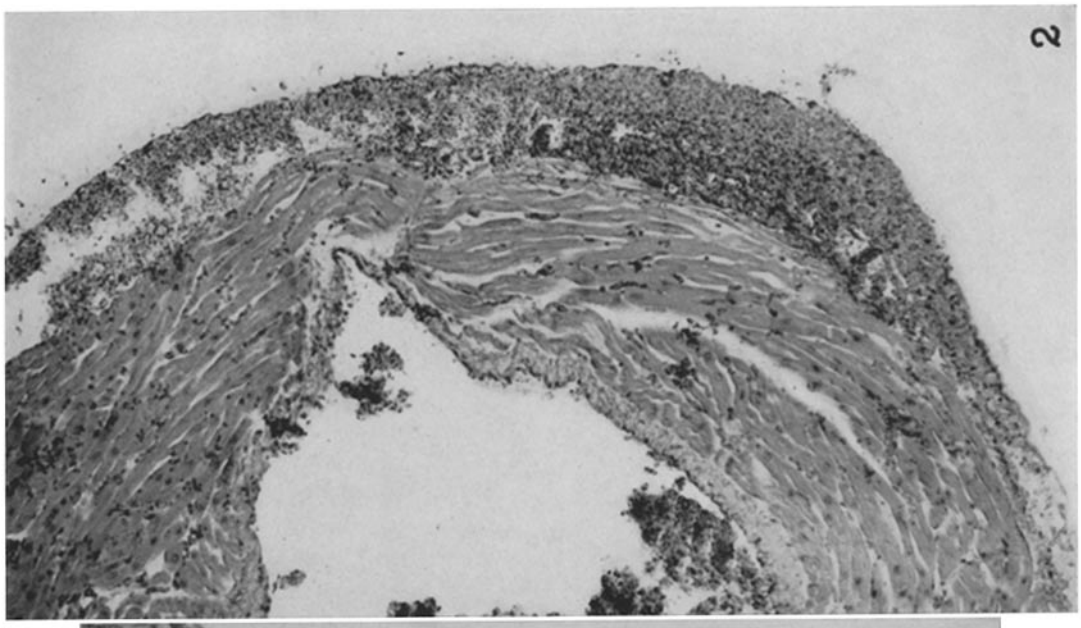
EXPLANATION OF PLATES

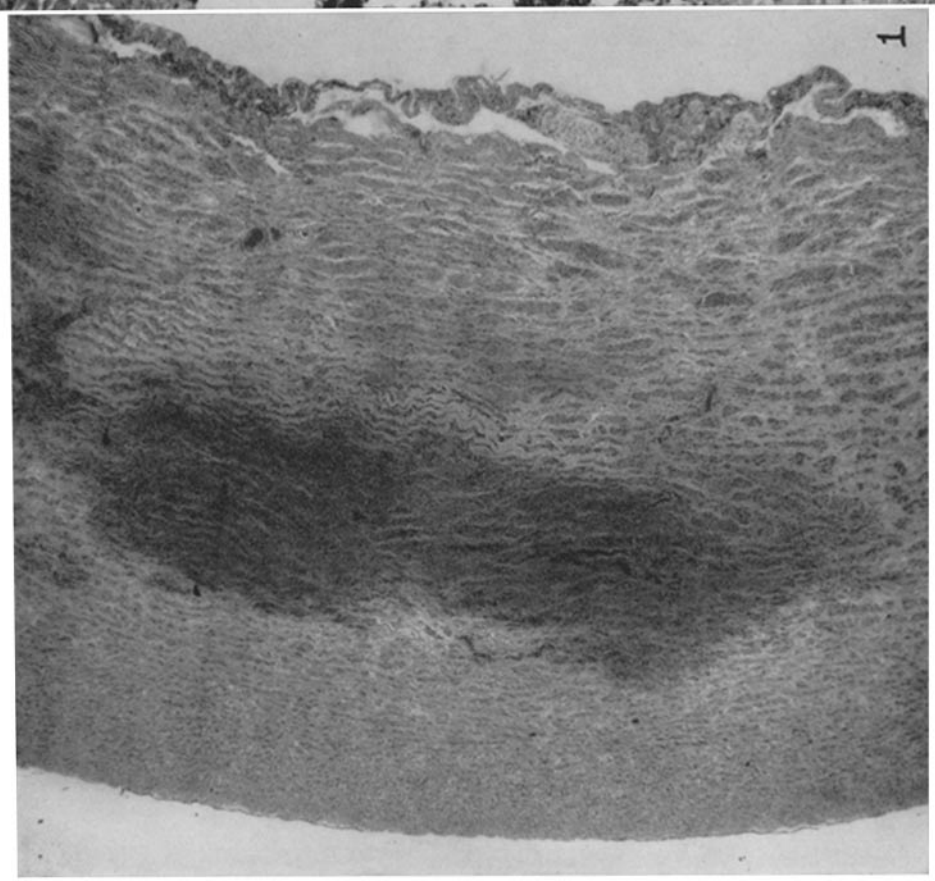
The photographs were made by Mr. R. F. Carter.
Hematoxylin and eosin were the stains used throughout.

PLATE 7

FIG. 1. Deer 2-3—dead 2nd day of illness. Section of arch of aorta showing massive hemorrhage infiltrating the strands of elastic and muscle tissue of the media.

FIG. 2. Deer 2-2—dead 1st day of illness. Section of tip of apex of ventricle showing accumulation of red blood cells separating the pericardium from the underlying heart musculature.



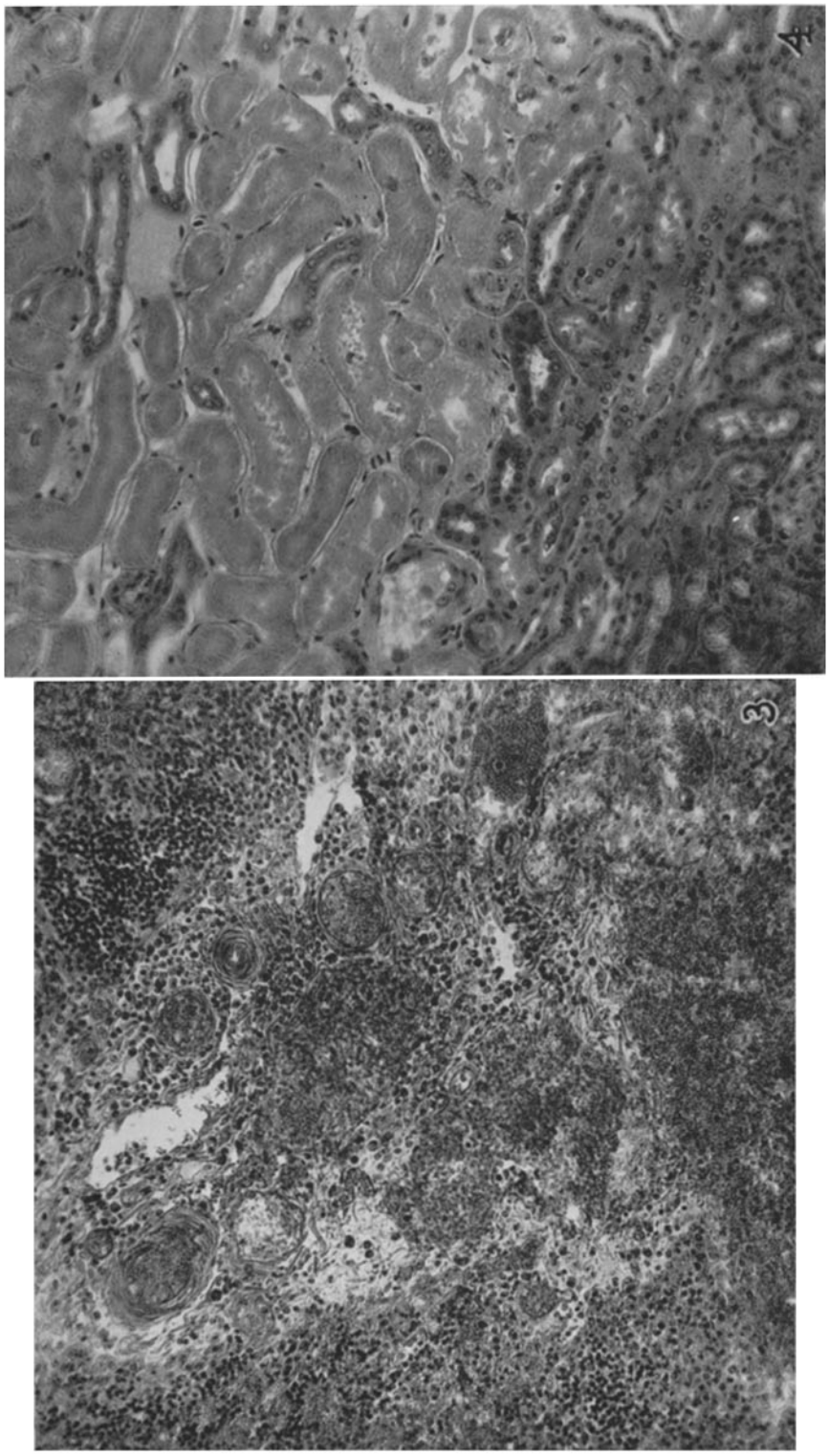


(Shope *et al.*: Epizootic hemorrhagic disease of deer)

PLATE 8

FIG. 3. Deer 1-7—dead 1st day of illness. Section of thymus showing dilated, blood-filled vessels, and hemorrhages into the parenchyma of the gland.

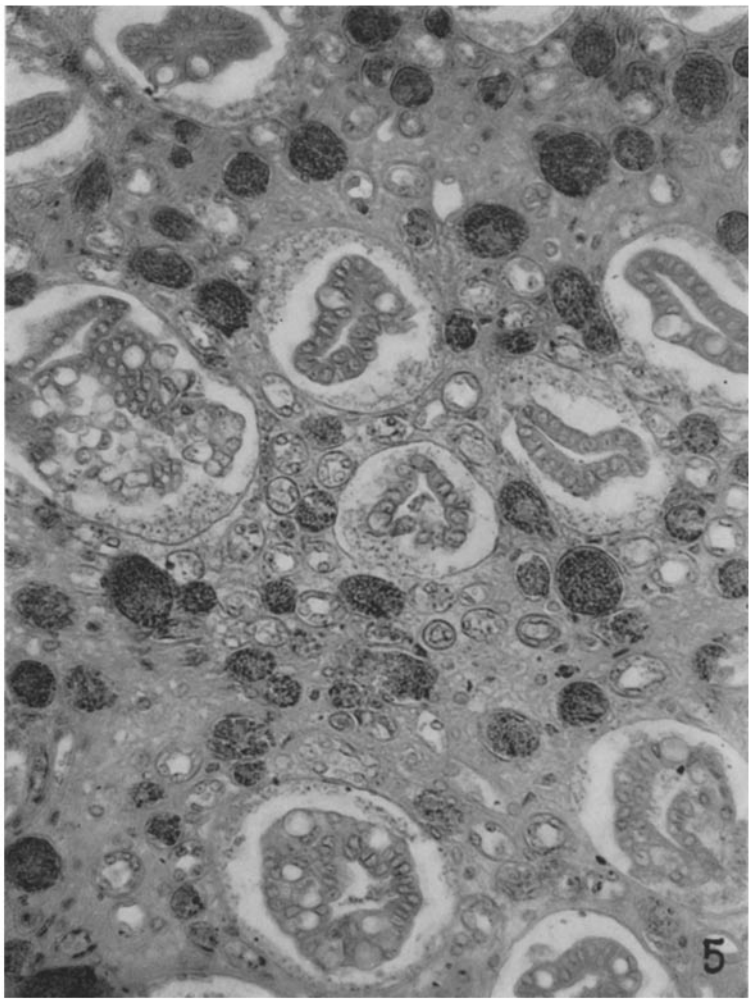
FIG. 4. Deer 2-2—dead 1st day of illness. Section of kidney showing necrotic renal tubules adjacent to some that appear relatively normal.



(Shope *et al.*: Epizootic hemorrhagic disease of deer)

PLATE 9

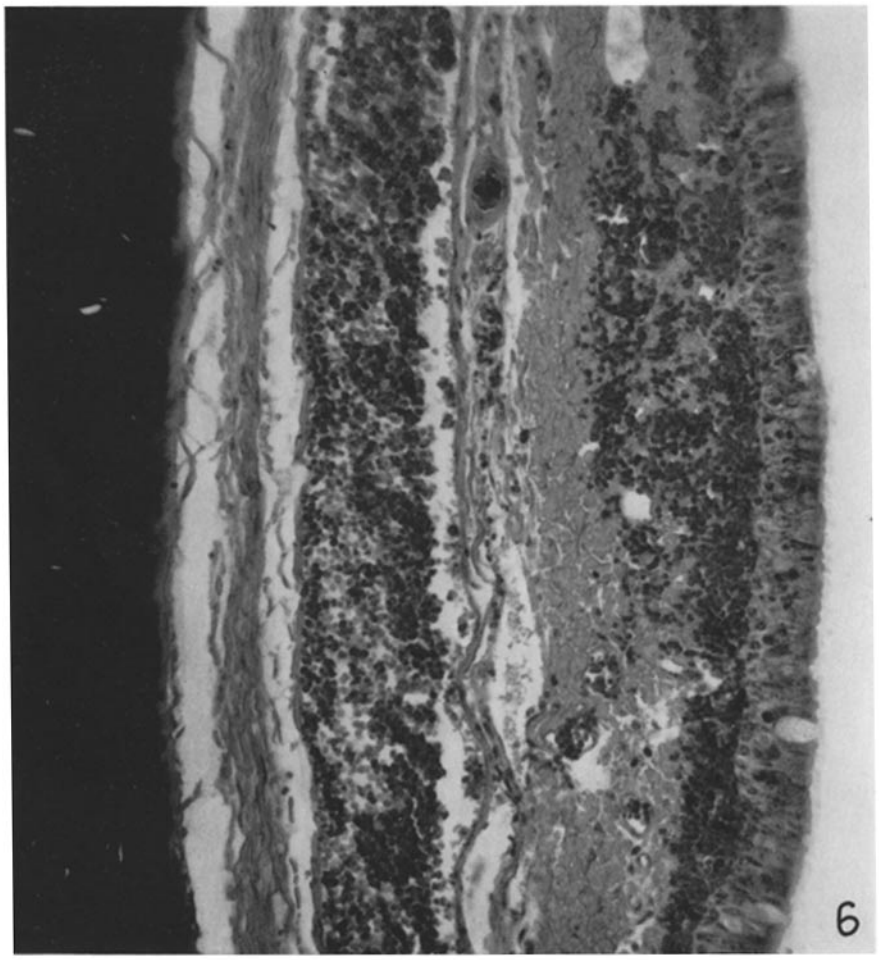
FIG. 5. Deer 2-2—dead 1st day of illness. Section of medulla of kidney showing distended blood vessels packed with red blood cells. The terminal collecting tubules are collapsed and many of the lining cells appear vacuolated.



(Shope *et al.*: Epizootic hemorrhagic disease of deer)

PLATE 10

FIG. 6. Deer 1-7—dead 1st day of illness. Section of trachea showing an area of submucosal hemorrhage and a distended blood vessel packed with red blood cells.



(Shope *et al.*: Epizootic hemorrhagic disease of deer)