TABLE I.

	Wt	Liver wt	Peroxide content per unit wt of liver
Control animals	222.0	6.84	100.1*
	315	8.90	100.0*
Treated "	219	6.80	102.0
	212	7.20	97.0
	384	10.8	97.4
	282	8.4	100.1
			Avg 99.1%

* Measured against an unfasted, untreated control rat of similar weight.

3 hours the animals were dispatched by a sharp blow to the head and the liver quickly isolated and weighed in ice cold distilled water. The tissue was minced and homogenized in a teflon pestled apparatus in 4 volumes of distilled water at 0°C. The homogenates were quickly frozen in an acetone dry-ice mixture and stored at –30°C before analysis. Aliquots of homogenate were analyzed for lipid peroxide by the thiobarbituric acid method(4).

Results and discussion. The results appear in Table I. The peroxide content of livers of untreated, non-fasted animals is arbitrarily set at 100%. There is no difference in control and experimental animals in regard to the amount of lipid peroxides in hepatic tissue at the 3-hour time period, when there are demonstrable morphologic alterations in the ergastoplasm and when there is clearly demonstrable depression of protein synthesis. It appears, therefore, that lipid peroxides do not play a significant role in the early phase of carbon tetrachloride intoxication.

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Isolation of a Virus from Infectious Bovine Kerato-Conjunctivitis. (27704)

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Infectious bovine kerato-conjunctivitis ("pink-eye") has a world-wide distribution. Outbreaks of the disease have been reported in India, South Africa, Holland, Canada, England and the United States(1). In one of the earliest detailed investigations of the disease, a classical description of its symptoms was presented(2). In this investigation, isolation of a pleomorphic bacillus from affected eyes was reported, but cultures of the organism failed to reproduce the disease in eyes of healthy cattle(2). Failure to induce the disease in cattle by inoculation of cultures of various types of organisms isolated from cases of the disease has been described(1). The possibility of Rickettsiae being associated with the disease has also been suggested(3). Infectious bovine rhinotracheitis virus (IBR) may cause changes of the ocular and palpebral conjunctiva, whereas symptoms of infectious bovine kerato-conjunctivitis (IBKC) are associated with changes of conjunctiva and cornea, which sometimes lead to loss of eye(2, 4,5). The rapid spread of IBKC through herds of susceptible cattle suggested a viral etiology of the disease. The present study was undertaken in an attempt to determine whether a virus was involved in the origin of this disease.

Materials and methods. Eye tissue obtained from cattle with acute infectious kerato-conjunctivitis was used as starting material.* Affected tissue was removed from eyes of infected cattle and shipped by a method previously described(6). Twelve le-

*Obtained through courtesy of Dr. C. Lindley, Dept. of Animal Husbandry, Mississippi State Univ., State College.

sions were received and pooled into 3 groups. The lesions were minced, ground with sand and suspended in 5 ml Hanks' balanced salt solution. Cell suspensions were centrifuged at 900 $\times$ *g* for 30 minutes at 4°C, the supernates removed and filtered through Millipore filter membranes (0.47 μ a.p.d.). The filtrates were inoculated onto monolayer cultures of embryonic bovine lung cells. Inoculated cultures were maintained at 38°C in T-30 culture flasks with Eagle's basal medium without serum.

Virus titrations and serum-neutralization tests were carried out in Sykes-Moore tissue culture chambers(7). Serum and virus dilutions were made using separate pipettes for each dilution. Antisera against a number of known bovine viruses were obtained from different sources.† IBR virus‡ was used as control agent. Fluid from tissue cultures, showing cytopathic effect and diluted to contain 6 logs of virus per ml, was used for bioassays in less than 6-month-old calves and in yearlings. The fluid was inoculated pernasally in 2-5 ml quantities.

Results. Inoculation of original cell-free material from each of the 3 pools onto monolayer cell cultures of fetal bovine lung cells was followed within 72 to 96 hours by massive cell destruction (Fig. 1-4). Whenever this cytopathic effect (CPE) was seen, tissue culture fluid was removed, and its cell-free filtrate in a dilution of 10^{-4} was inoculated onto fresh monolayer cultures of fetal bovine lung or kidney cells. Cytopathic effect occurred regularly between 60 to 72 hours during 5 consecutive passages of similarly prepared material. Material from fifth passage was titrated using Sykes-Moore chambers(7) and roller tubes. After 5 days incubation at 38°C, CPE was seen in chambers inoculated with material in dilutions up to 10^{-11}, while tube cultures showed CPE with the same material, diluted up to 10^{-8}. Tissue culture chambers permit examination of cells at high magnifications, hence the apparent difference in CPE titer between chamber and tube cultures. Starting material in dilutions from 10^{-1} to 10^{-6} gave CPE after 48 hours, and in higher dilutions (10^{-6} to 10^{-10}) after 72 hours. Tissue culture material at high dilutions induced nuclear changes in some cells of the chamber cultures, which could be seen by fluorescence microscopy following staining with Coriphosphine O. The changes were characterized by margination of chromatin and greatly increased fluorescence of nuclei (Fig. 5 and 6). In some cells, increase in DNA staining material was localized in round masses in the nucleus.

Activity of the agent in tissue culture material was not affected by treatment with an equal volume of ethyl ether for one hour at 38°C. It was preserved for 9 months at −70°C, with little loss following storage at 4°C for 3 weeks. Rapid loss of activity occurred when the same material was stored at room temperature (20°C) for one week or heated at 56°C for 30 minutes. The agent present in tissue culture fluid does not hemagglutinate red cells from chickens, mice, hamsters, guinea pigs, rabbits, bovines, sheep or man. Cultures treated with 5 μg of 5-Fluorodeoxyuridine or 5-Bromodeoxyuridine/ml of medium at time of inoculation with the agent remained free from CPE. Similar cultures treated with 5 μg of 5-Fluorouracil/ml of medium at time of agent inoculation showed characteristic CPE at the usual time. Addition of any of these compounds to cultures 3, 5, or 15 hours after viral infection failed to affect activity of the agent. All the findings indicate that the agent is a virus which contains DNA.

The virus multiplies and produces CPE in cultures of embryonic bovine lung, kidney, corneal cells and of human embryo cells.

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‡ Obtained through courtesy of Mr. L. Drehle, Colorado Serum Co., Denver, Colo.

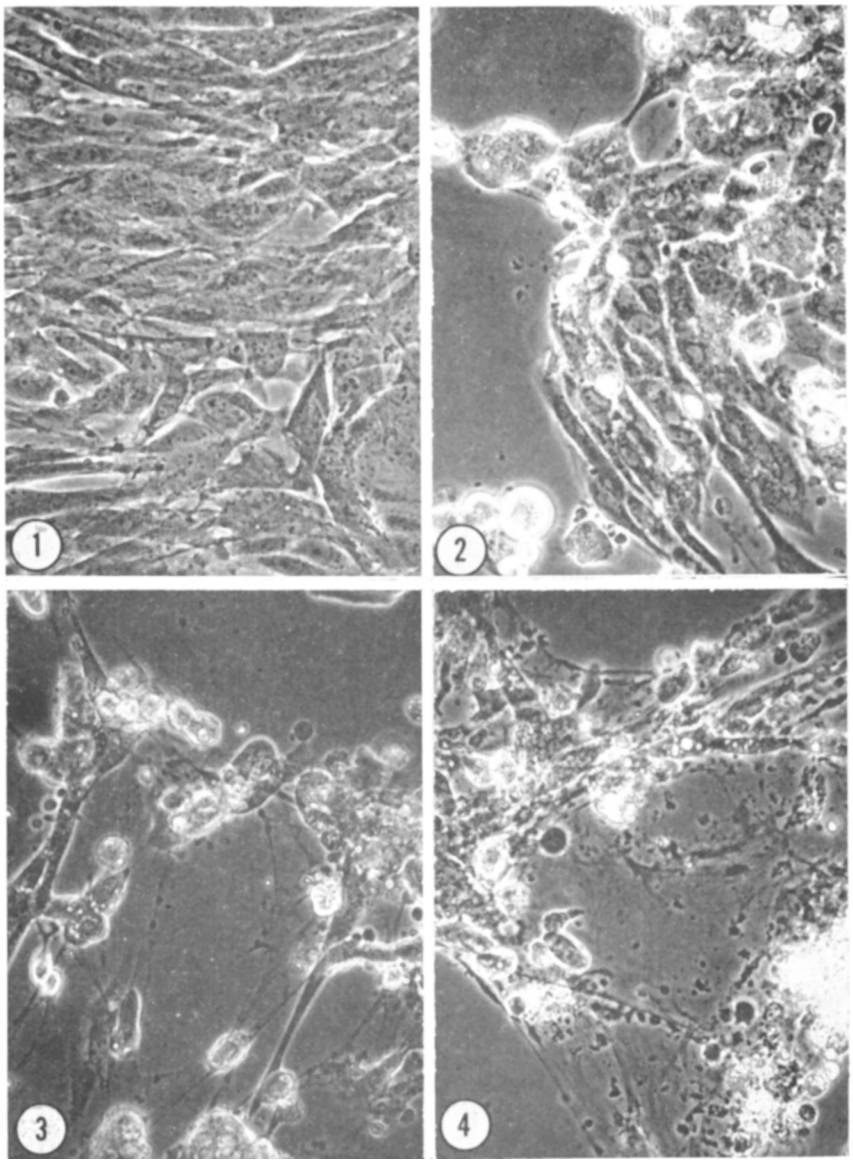

FIG. 1. Appearance of uninoculated control culture of embryonic bovine lung cells. Phase contrast photomicrograph. 600 ×.

FIG. 2. Embryonic bovine lung cells inoculated with IBKC virus at 10^{-8} dilution, showing early CPE, 60 hr after inoculation. Phase contrast photomicrograph. 600 ×.

FIG. 3 & 4. Embryonic bovine lung cells inoculated with IBKC virus at 10^{-11} dilution, showing massive CPE 72 hr after inoculation. Phase contrast photomicrographs. 400 ×.

There is no evidence of viral activity in cultures of L cells. The virus is sedimented by centrifugation at 105,000 × *g* for 2 hours, as infected tissue culture preparations are rendered non-infective by this method.

In neutralization tests carried out in Sykes-Moore culture chambers, antisera (bovine and rabbit) from different sources against a number of bovine viruses failed to neutralize CPE of the agent isolated from cases of IBKC (Table I). The tests were carried out using both the neutralization-index method(8) and the fixed virus falling serum concentration method. By both methods, all tested sera at 1:2 or higher dilution failed to neutralize the virus (50 $TCID_{50}$). As IBR virus has been

found to cause eye symptoms(4), all neutralization tests were carried out in parallel using both the newly isolated virus and IBR virus. Sera from animals with IBKC following experimental infection with tissue culture passaged virus were the only sera found to have neutralizing antibodies against the agent *in vitro*. Titers of sera varied from 1:40 to 1:320 in cattle with mild disease or more severe disease symptoms. The sera showed no

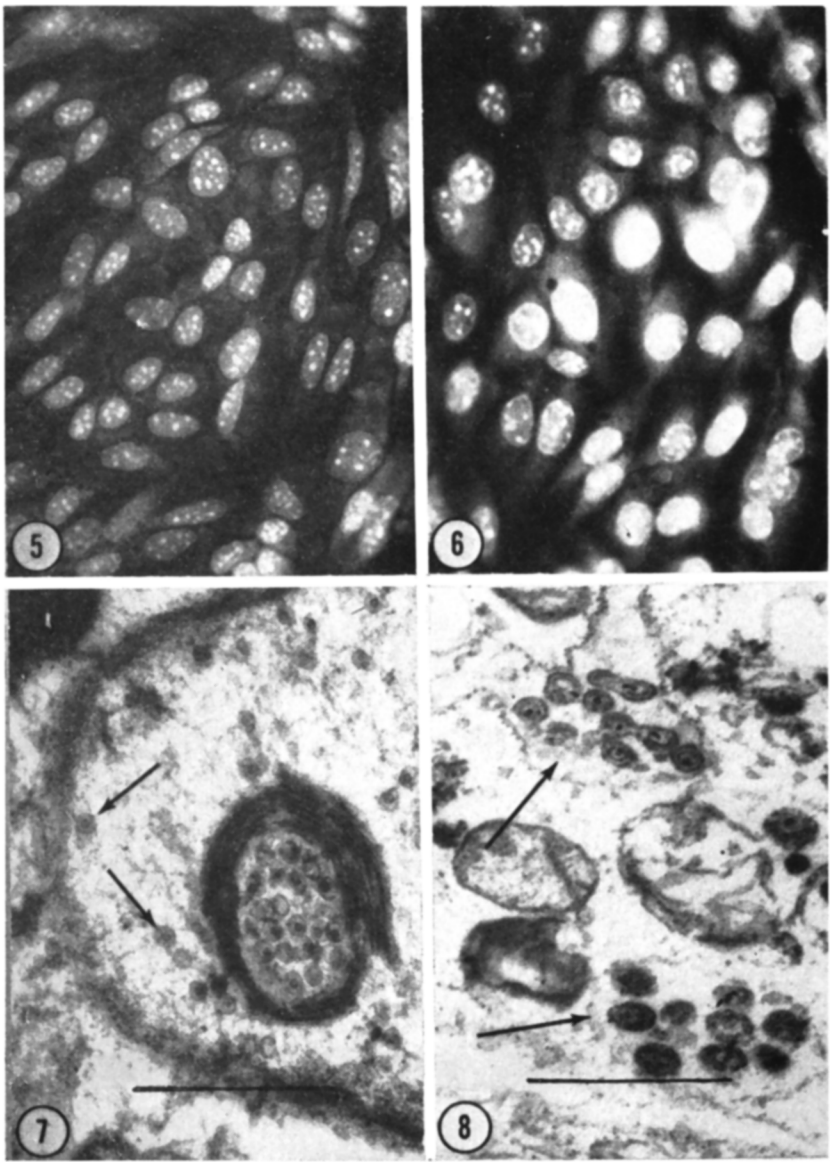

FIG. 5. Control uninoculated bovine embryo lung cells, stained with Coriphosphine O. Fluorescence photomicrograph in black and white. 400 ×.

FIG. 6. Bovine embryo lung cells infected with the agent, stained with Coriphosphine O. Marked increase in DNA fluorescence in nuclei of some cells. Fluorescence photomicrograph in black and white. 400 ×.

FIG. 7. Part of nucleus of infected bovine embryo lung cell. Scattered virus particles (arrows) present in nucleoplasm, also surrounded by characteristic membranous structures. Electron micrograph. 28,000 ×.

FIG. 8. Part of cytoplasm of infected bovine embryo lung cell. Characteristic virus particles (arrows) present among cellular constituents. Electron micrograph. 28,000 ×.

TABLE I. Antisera from Various Sources* tested with Negative Results for Neutralizing Activity *in vitro* Against Virus Isolated from IBKC Tissues.

Type of antiserum	No. tested Calf	Rabbit	Type of antiserum	No. tested Calf	Rabbit
Enteric viruses			Respiratory viruses		
Bovine virus diarrhoea	4	3	IBR virus	4	6
Mucosal disease virus	6	1	Para-influenza 3	1	3
Unknown Nebraska agent	1	–	Bovine respiratory virus	–	4
Klein adeno-virus	–	1	Pneumo-enteritis virus	–	4
Other viruses			Control preinoculation sera	4	5
Pustular vulvovaginitis virus	–	1			

* See † footnote in text.

neutralizing activity against IBR virus in tissue culture.

Biological tests of tissue culture passaged virus (Passage 11) were carried out on 6 six-month-old calves and 12 year-old heifers (Passage 20) (Table II).§ In both tests, 2-5 ml of tissue culture fluid, diluted to a CPE titer of 10^{-6} in chambers, were used as inoculum. The virus was introduced pernasally from a 5-ml syringe. In the first group, 3 out of 6 calves developed symptoms of acute IBKC 4 days following inoculation. Unfortunately, in this group pre-inoculation serum samples were not obtained. Serum samples were obtained in this group of animals 14 days following inoculation of the virus. Two of the inoculated calves with symptoms of disease had a neutralizing antibody titer of 1:40. The post-inoculation serum from third animal with symptoms was found toxic to cells in serum control cultures. Two calves which failed to develop the disease showed virus-neutralizing titers in their sera of 1:80 and 1:160 (Table II). Serum of the remaining animal without symptoms was found toxic. It is possible that the calves without symptoms but showing neutralizing antibody titers had a previous history of disease that could not be ascertained as pre-inoculation serum samples were not available. In the second group (heifers), pre-inoculation serum samples were free from neutralizing antibodies both against the agent and against IBR virus when tested in tissue culture. Five out of 12 inoculated animals showed disease symptoms of varying severity. Post-inoculation serum samples collected 11 days after occurrence of symptoms (before the animals had to be shipped) showed virus-neutralizing antibody in tissue culture varying in titer from 1:40 to 1:320. Three out of 5 animals showed more severe disease and in these animals the neutralizing antibody titer could be related to the severity of disease. Two animals with minimal symptoms had no neutralizing antibodies in their sera. No neutralizing antibodies against IBR virus in tissue culture

§ Cooperation of Dr. Charles Lindley, and Dr. David C. Ballard in these tests and permission of the SMS Ranch owners to use some of their cattle for these tests, is gratefully acknowledged.

TABLE II. Biological Studies of Tissue Culture Passaged Virus Isolated from "Pink-Eye" Lesions and Inoculated Pernasally into Cattle.

Animal No.	Severity of disease*	Neutralizing antibody titer† Pre-inoculation	Post-inoculation
M 1	4	Not available	1:40
M 2	3	" "	1:40
M 3	3	" "	Toxic
M 4	0	" "	1:80
M 5	0	" "	1:160
M 6	0	" "	Toxic
S 1	3	0	1:320
S 2	0	0	0
S 3	2	0	1:80
S 4	1	0	0
S 5	0	0	0
S 6	2	0	1:40
S 7	0	0	0
S 8	0	0	0
S 9	0	0	0
S 10	1	0	0
S 11	0	0	0
S 12	0	0	0

* Severity of disease — Classical pink-eye affecting both eyes, 4; Classical pink-eye affecting one eye, 3; Moderately severe lacrimation, 2; Mild lacrimation, 1.

† Neutralizing antibody tests were carried out in tissue culture. Sera were not tested in dilution higher than 1:320.

could be detected in any of the post-inoculation serum samples. Serologic studies are in progress of coded sera from a large number of cattle many of which have had classical "pink-eye".‖

Preliminary electron microscope studies of fetal bovine lung cells grown *in vitro* and infected with the virus have shown characteristic nuclear changes of a type not previously described (Fig. 7). These studies have demonstrated a highly complex cycle of viral multiplication in the nucleus (appearance of different size granules, formation of concentric membranous structures) which is followed by further development of virus in the cytoplasm. The nuclear particles are composed of a nucleoid (440 Å) surrounded by a single membrane and the cytoplasmic particles have a double membrane. Initial measurements of virus size indicate a particle diameter varying from 890 Å to 1250 Å in the nucleus and of 1400 Å average diameter in the cytoplasm (Fig. 8). These findings will be described in greater detail elsewhere.

Discussion and summary. A virus has been isolated from biopsy material from eyes of cattle with acute infectious bovine kerato-conjunctivitis. No serum samples from these animals were available for testing against the virus. The virus is cytopathogenic for cells of bovine and human origin; ether resistant; relatively heat labile; does not hemagglutinate red blood cells from chickens, mice, hamsters, guinea pigs, rabbits, bovines, sheep, or man. Fluorescence microscopy of infected cells stained with Coriphosphine O and studies on the influence of 5-BUDR, 5-FUDR, and 5-FU on virus multiplication indicate that it is a DNA virus. Antisera (bovine or rabbit) against enteric, respiratory and other bovine viruses have failed to neutralize cytopathogenic activity of the virus. Per-nasal instillation of virus-containing tissue culture fluids has induced classical infectious bovine kerato-conjunctivitis of varying degrees of severity in 8 out of 18 inoculated cattle. It is of interest that 7 out of 12 heifers have failed to respond clinically and immunologically to inoculation of the virus which may be due to failure of inoculation rather than to immunity as pre-inoculation serum samples had no neutralizing antibodies. Sera from animals with experimentally induced acute kerato-conjunctivitis have neutralized the cytopathic effect of the virus. Electron microscope studies have demonstrated that the virus induces nuclear changes of a type not previously described in tissue culture cells infected with any known virus. Recent electron microscope studies(9) of infectious pustular vulvovaginitis virus (IPV) and IBR virus in tissue culture have confirmed previously published studies on IBR virus(10,11). There appears to be a similarity in particle size and structure between these viruses and the agent isolated from eyes of cattle with IBKC. However, there appears to be a difference between nuclear changes observed in cells infected with the agent isolated in the present study (formation of whorls, see Fig. 7) and those observed in cells infected with IBR(10,11) and IPV (9). The findings indicate that this virus might be causatively related to acute infectious bovine kerato-conjunctivitis ("pink-eye"), should further animal inoculation studies confirm these preliminary observations.

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‖ Obtained through the courtesy of Dr. R. L. Sweat, Dept. of Veterinary Med., Coll. of Agric., Univ. of Nebraska, Lincoln.

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Pressure Gradients in the Splanchnic Bed of the Monkey During Hemorrhagic Shock.* (27705)

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In reviewing the comparative physiology of experimental shock, Zweifach(1) has called attention to the paucity of information for primates. The imperative need for treatment of the human in shock has greatly limited the study of physiological mechanisms in this species, and very few data are available in other primates. The purpose of the present investigation was to gain information on the behavior of the splanchnic circulation of the monkey during a standardized hemorrhagic shock procedure.

Methods. Eight squirrel monkeys (*Chrysothrix sciurea*) of both sexes, averaging 635 g in weight were used. Under pentobarbital anesthesia (35 mg/kg, I.P.), laparotomy permitted placement of a fine-bore polyethylene catheter into the portal vein *via* an intestinal vein tributary. *Via* the jugular vein, another catheter was positioned at the juncture of the hepatic veins and the inferior vena cava. A carotid artery was also catheterized. Pressures were simultaneously recorded with Statham transducers on an Offner recorder. Zero pressures were corrected to the level of the thoracic vena cava at the conclusion of the experiment. In 2 experiments, portal blood flow was measured with a low resistance bubble flow meter after adequate heparinization. Heart rate was measured from a lead II ECG tracing. Respiratory rate was recorded with a force transducer attached to the thorax, or computed from respiratory variations in the venous pressure tracings. Rectal temperature was continuously monitored, and body temperature was kept as constant as possible with the aid of a heat lamp.

* Supported by U. S. Public Health grant.

The hemorrhagic shock procedure was to bleed from an artery (carotid or femoral) into an overhanging reservoir whose height was adjusted to maintain the mean arterial blood pressure at 40 mm Hg. This was kept at the desired level until the animal had taken back 30% of the bleeding volume from the reservoir. At this time, the remainder of the blood was returned, and the post-transfusion events were followed until demise of the animal.

Results. Duration of the oligemic phase averaged 2 hours, and the normovolemic phase following transfusion 2 hours, 25 minutes (for range of values, see footnote, Table I). Results are given in Fig. 1 and Table I in relation to percentage duration of the oligemic and normovolemic phases.

Effects of hemorrhage: Oligemic phase. Fig. 1 shows the average trends obtained in 6 animals in which portal flow was not measured. The top curve shows the bleeding volume, which at the maximum averaged 1.9% of body weight. Below the curve depicting the arterial pressure appear the related trends in portal vein and inferior vena cava pressure. Following hemorrhage, the respective pressures decreased to 37, 58 and 25% of control.

The fourth panel of the figure shows the change in R_M/R_H ratio (mesenteric/hepatic vascular resistance). Changes in this ratio give insight to the relative resistance changes in the splanchnic bed. This was achieved by the method of vertical analysis employed by Wiggers, Opdyke, and Johnson(2) in the canine splanchnic bed during hemorrhagic shock. R_M is taken as $(P_A - P_{PV})F_1$, where