Experimental infection of pregnant mares with equine arteritis virus

by

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INTRODUCTION

Equine viral arteritis has been associated with outbreaks of abortion in equine breeding farms. The pattern of the disease is characterized by influenza-like signs in all horses of the herd and abortion but with low mortalities in pregnant mares. Necropsies of animals dying from the disease and studies of lesions in experimentally infected animals reveal fibrinoid necrosis in the media of small muscular arteries. No description on pathology of uterus and placenta in association with abortion has been published. This study has been undertaken to investigate abortion by equine arteritis virus in experimentally infected pregnant mares.

LITERATURE REVIEW

Brief historical review

An outbreak of abortions associated with respiratory signs which occurred in 1953 in Bucyrus, Ohio, led to the identification of equine viral arteritis (EVA). The viral, clinical, and pathological features of this new condition were reported in a series of articles from scientists in Lexington, Kentucky, in 1957 (7,13,14,24). Because of the necrotizing arterial lesions in infected mares, the name of equine viral arteritis was proposed.

Serum neutralization tests after equine arteritis virus viral infection, now an established cause of abortion in horses (14), indicate no increase in neutralizing antibodies against equine rhinopneumonitis (ERP) virus. Arteritis virus does not stimulate formation of complement fixing antibodies against rhinopneumonitis virus. In addition, the two viruses do not confer any reciprocal immunity to infected animals. Therefore, EVA and ERP are considered as two different diseases, with two distinct etiologic agents (13).

Clinical data

In two horse breeding farms where EVA occurred, signs were seen in all groups of animals 7 to 17 days after first exposure. About 50% of pregnant mares aborted during the first two weeks after the first signs of illness or without any other clinical sign. Barren mares and fillies were mildly affected, 7 to 8 stallions had edema of the sheath and scrotum. Fever was present in all cases, beginning 12 to 96 hrs after

exposure and ending in 4 to 9 days. Morning remissions occurred during the first 2 to 3 days of fever (7).

Other clinical signs were quite variable. Seventy-five percent of animals had lacrimation, conjunctivitis, palpebral edema, nasal discharge, edema of the legs and abatement. Anorexia that persisted 1 to 5 days was seen in the more severe cases. Fetuses were expelled with their membranes and little or no autolysis was seen. Petechial hemorrhages on the pleura and upper respiratory tract were occasionally present (14).

Horses had severe leukopenia with total white blood cell count reductions to 52% for neutrophils and 65% for lymphocytes. The average duration of leukopenia was 8.8 days, the lowest counts were found 5.6 days after inoculation. A biphasic response with secondary leukocytosis and neutrophilia was observed in older horses (7).

Lesions

Gross lesions and histopathology in 28 horses, including 8 pregnant mares, were subcutaneous edema, edema of the digestive tract and lungs, and hemorrhages in the heart and spleen. In pregnant mares, the uterine wall was edematous and, in some cases, a dead fetus was found (24). Microscopic lesions of the circulatory system were restricted to small muscular arteries. Fibrinoid necrosis of the media appeared to be the earliest change. It was followed by leukocytic infiltration of the adventia and perivascular edema. Small and large intestine, lungs, and

various tissues had the same type of lesions. No arterial lesion could be demonstrated in fetuses, although their tissues were highly infective (24).

Crawford and Henson have shown that the histologic lesions of EVA should be more accurately described with the term panvasculitis versus arteritis, in which lymphatics, veins and arteries are equally affected (10).

Ultrastructurally, the most striking lesions were found in endothelial cells of capillaries, veins and arteries. They included cell swelling, cisternal dilatation, enlargement of lysosomes and mitochondria. Characteristic changes due to the presence of the virus in the cell included intracisternal ribosome-like particles, intracytoplasmic dense particles, and membrane-tubule formations. Virions, with an average diameter of 58 nm, were present within cytoplasmic vacuoles. The typical lesion of EVA, necrosis of the media of the small muscular arteries, was attributed to anoxic changes since viral particles were not identified in smooth muscle cells (18).

Experimental disease

Subcutaneous inoculation of seven pregnant mares with spleen and lung suspensions from an infected fetus resulted in death of 4 animals between the 5th and the 7th day postinfection (24). At necropsy, one fetus dead *in utero* for about 48 hrs was found. The three other fetuses were still alive during the terminal phase. Three horses survived but aborted on the 7th, 11th and 13th day postinoculation respectively. Two

fetuses were autolyzed, one did not present any sign of postmortal change. Aborted fetuses had subcutaneous edema, excessive peritoneal fluid and slight edema of the lung (13).

Pathogenesis

Using immunofluorescence, the distribution of EVA virus was demonstrated. After experimental infection by aerosol, the first target for virus replication was the pulmonary macrophage. Subsequently, virus spread to the regional lymph nodes and then was disseminated systemically. Major sites of viral replication were macrophages and endothelium, including arteries, veins, lymphatics, and endocardium. Cells of the tunica media were affected later in the course of the disease. Mesothelium and endothelium of certain organs were other sites of viral multiplication (31).

Endothelial necrosis and disruption of internal elastic lamina led to a leakage of fluid through the media. The thickness of the media in large size arteries did not allow the virus and phlogogenic macromaterial to cross the vessel wall. This accounted for the lack of media necrosis in muscular arteries over 2 mm diameter (10).

Antibodies did not alter the development of lesions in immunosuppressed horses. From successive biopsies in horses infected with EVA virus it was showed that once the initial lesion is induced, veins and lymphatics recovered by 10 days and complete regeneration of muscular arteries occurred by 2 months. This situation is not compatible with long term host-agent relationship. EVA was considered as a unique

disease, although the resulting lesions were comparable with other arterial necrotizing diseases of man and animals (21).

Virus

EVA virus replicates in several primary and secondary cell culture systems. Propagation on primary equine kidney cells of virus isolated from spleen tissue of aborted fetuses produces necrosis of the cell culture. Kidney cells from hamster, rabbit, pig, cat, African green monkey (VERO), baby hamster, cynomolgus monkey (Jinet), as well as hamster lung cells are susceptible to EVA virus (23).

Passages of EAV in tissue culture reduces its pathogenicity. Horses were infected with EVA virus propagated in equine kidney. The 7th passage virus killed horses in 7 days with typical signs and lesions of EVA. Tenth passage virus infection resulted in a marked clinical disease with fever and leukopenia followed by recovery. The 25th passage virus induced mild clinical disease. Attenuated virus over 25 passages failed to produce any clinical sign among infected horses. Serum from a convalescent horse infected with 10th passage completely inhibited the CPE on cell cultures (30). EAV was present in nasal secretions.

In experimental infection of horses with field isolate, virus was found in nasal exudate from day 1 to day 14 after inoculation (31). In addition, virus is present in lung, spleen, liver, heart blood, pleural, abdominal, and pericardial fluids in the fatally infected horses. The presence of virus in the nasal exudate became less apparent with

succeeding passages on equine kidney cells. Nasal swabs collected from horses exposed to the 116th passage yield no virus (29).

Magnusson et al. studied the morphology of EVA virus on hamster kidney cells. They describe 50 nm enveloped particles with a 25-30 nm diameter central core. A possible relation between EVA virus and group A arboviruses was suggested (27). Burki pointed out similarities of this virus with hog cholera, bovine virus diarrhea, and human hepatitis agents (8). The presence of a ribonucleic core was established by specific ferritin-tagged antibodies (6).

The EVA virus genome consists of a single stranded RNA, with a molecular weight of about $4x10^6$ daltons. With reference to a well-known togavirus, Semliki forest virus (SFV), EVA virus has been definitely classified in the family togaviridae (45). Molecular weights of its three structural proteins do not allow to include EVA virus in established genera. The assignment of a specific taxonomic genus including EVA virus and lactic dehydrogenase virus of mice has been proposed (47).

Vaccination

Inoculation of horses with virus attenuated by 131 passages on horse kidney cells followed by 50 to 111 passages on rabbit kidney cells results in immunization.

The vaccine is safe for any kind of horse, except for mares in late gestation. Evidence of fetal infection and subsequent abortion have been observed (15,28,25).

Epidemiology

In the United States, less than 3% of the horses had specific antibodies against EVA virus in 1972. In Switzerland, 5.7% of the horses, and in France, an average of 15%, were serologically positive. European authors consider the problem of inapparent virus carriers, owing to the lack of correlation between these immunological data and the clinical records in these countries (19,34).

STATEMENT OF PROBLEM AND OBJECTIVES

Abortion is the most important clinical manifestation of equine viral arteritis in natural outbreaks. In herds without pregnant mares, the disease appears as a mild respiratory infection. Lesions found in EVA are systemic, with necrosis of blood vessel walls in various organs and parenchyma.

This study was undertaken to describe the lesions of fetus, placenta, and maternal genital tract after equine viral arteritis abortion; to correlate the lesions with abortion, and to clarify the relation existing between clinical signs and lesions in EVA.

MATERIALS AND METHODS

Horses infected with EVA virus

Animals Eight Shetland ponies, 7 for inoculation and 1 control, were used for this study. The animals came from an area known to be free of equine viral arteritis.

Virus The virus used for this study was the original Bucyrus strain provided by Dr. W. C. McCollum. Ten percent suspension of virus from the spleen of a horse which died from EVA was used for intravenous inoculation.

Clinical data and samplings The animals were kept individually in separate rooms and examined daily for one week before inoculation. Temperature was recorded daily, starting 1 to 4 days prior to inoculation and twice a day after inoculation continuing to death. Fifteen ml blood was collected daily from the jugular vein in vacuum tubes containing 7.2 mg EDTA (VACUTAINER^{R} Becton, Dickinson). Packed cell volumes (PCV), total white blood cells (WBC) and total red blood cells (RBC) were determined. A second sample was kept at 37^{o} C for platelet counts. WBC and RBC determinations were done by electronic cell counter (Coulter Counter Mod. B, Coulter Electronics). Platelet counts were done by phase microscopy with a hemocytometer (0.1 mm deep hemocytometer, Bright Line^{R} American Optical) with a 1% solution of whole blood in ammonium oxalate. PCV was determined on a microcapillary reader (Damon^{R} IEC) after centrifugation for 5 minutes (International microcapillary centrifuge, Mod MB, IEC). Three determinations were done on each sample for

WBC, RBC, PCV, and platelets. Plasma was collected from the jugular blood in heparinized vacuum tubes (VACUTAINER^{R} Becton, Dickinson) and, after centrifugation, stored at -70^{o} C. Levels of progesterone, prostaglandin $F_2\alpha$($PGF_2\alpha$), 13,14-dihydro-15-ketoprostaglandin $F_2\alpha$(13-14 $PGF_2\alpha$), lactic dehydrogenase (LDH), sorbitol dehydrogenase (SDH), ornithine carbamyl transferase (OCT), gamma glutamyl transpeptidase (GGTP), lactic acid (LA), blood urea nitrogen (BUN), glucose, chloride, phosphorus, sodium, potassium, magnesium, calcium, and creatinine were determined from stored serum, on every sample, for each animal. Progesterone was determined by radioimmunoassay in 0.5 ml plasma (I Progesterone Radioimmunoassay Kit, Diagnostic Biochem International Inc.). Prostaglandins were extracted and determined by the method of Baetz and Pier (4). The method reported by Strandjord and Clayson (44) was used for OCT determination. GGTP was determined by automated colorimetric method (40) and LA determination was performed by an automated enzyme fluorometric assay modified from Hochella (in(2)). BUN, glucose, chloride, phosphorus, LDH and creatinine were determined by AATM following the methodology supplied by the manufacturer (Technicon Instrument Corporation, Auto Analyzer Technical Methods). Sodium and potassium were determined in 1/200 dilution of plasma into a flame photometer (Beckman Model B with flame attachment, Beckman Instruments). Calcium and magnesium were determined in 1/25 dilution of plasma into an atomic absorption spectrophotometer (Model 290, Perkin-Elmer Corp.).

Necropsy, specimen collection, and processing Horses were killed during the agonal phase of the disease or, in one case, after abortion. Death was induced by an intravenous injection of pentobarbital (Nembutal^{R} Sodium, Abbott) and succinylcholine chloride 10% solution immediately followed by severing of the axillary blood vessels. Samples collected for virology, histology, electron microscopy and immunofluorescence studies are listed in Figure 1. Pieces of tissue for virus isolation were frozen in dry ice. Samples remained frozen at -60^{o} C until used for isolation procedure. Tissue samples were thawed and weighed in sterile petri plates. A 10% (W/V) suspension was made in a TenBroek tissue grinder with Eagle's MEM (minimum essential medium) as a diluent. It was centrifuged at 500 g for 20 minutes and the supernatant was collected. Serial 10-fold dilutions of supernatant were made with Eagle's MEM as diluent. A 0.2 ml sample of each dilution was pipetted onto a chamber of a 4-place chamber slide containing a monolayer of embryonic mule skin (EMS) cells. After 2 hrs at 37^{o} C, the inoculum was removed and the monolayers were rinsed. Maintenance medium was added and the cultures were incubated at 37^{o} C. After 20 hrs, chamber slides were rinsed with PBS (pH 7.6) and fixed for 5 minutes in acetone. Cultures which had no CPE at 1 week were passaged 2 times before being considered negative. Tissues selected for histology were fixed in 10% buffered formalin, placed in tissue capsules, dehydrated in graded alcohols, cleared in xylene, and infiltrated and embedded in paraffin using an autotechnicon (Autotechnicon^{R} Mod. 2A, Technicon), cut at 6 μm

	Organ	Histology	Electron microscopy	Immuno fluorescence	Virus isolation
Fetus	Blood	-	+	-	-
	Mes. lymph node	+	-	+	-
	Spleen	+	-	+	+
	Liver	+	-	+	-
	Kidney	+	-	+	-
	Lung	+	-	+	+
	Umbilicus	+	-	+	-
	Placenta	+	+	+	+
	Testes/Ovary	+	+	+	+
Mare	Blood	-	-	-	-
	Endometrium	+	+	+	-
	Ovary	+	-	+	+
	Spleen	+	-	+	-

Figure 1. Samples collected in pregnant mares and fetuses experimentally infected with EVA virus

and stained with hematoxylin and eosin (H&E). Masson's trichrome, Van Gieson, phosphotungstic acid hematoxylin (PTAH), and Gomori's aldehyde fuchsin method for elastin stains were also used on some samples. Frozen sections cut at 10 μm were stained with oil red O (ORO). One millimeter thick pieces of tissue were fixed in 2.5% glutaraldehyde for 2-3 hrs, rinsed twice in cacodylate buffer pH 7.4, post-stained in 1% osmium tetroxide for 2 hrs, dehydrated in graded alcohols, cleared in propylene oxide and polymerized in epoxy-resin (EPON 812, Shell Chemical Co.). Ultrathin sections were cut with an LKB 8801A ultramicrotome (LKB-produkter AB), stained with uranyl acetate and lead citrate and examined with a Philips 200 transmission electron microscope operated at 60 KV. For immunofluorescence, tissue specimens were immersed in embedding medium (2% methyl cellulose), placed on dry ice and subsequently frozen at -70° C. The age of fetuses was determined by measurements (5,16,35).

Immunofluorescence Serum globulin from a pony that had received multiple injections of tissue culture-adapted EAV was labeled with fluorescein isothiocyanate (FITC) and used for direct immunofluorescent stain (20). Labeled globulins (conjugate) were checked for reaction with equine infectious anemia virus (EIAV) antigen in an agar gel immunodiffusion test. Cells infected with equine herpesvirus-2 (EHV-2) were stained to check specificity of the conjugate. Conjugate was adsorbed with acetone-dried rabbit liver powder to remove non specific background staining before it was diluted 1 to 4 with PBS (pH 7.6) for use. Fixed cell monolayers were incubated with conjugate in a humid

chamber for 30 minutes at 37° C. Slides were washed 3 times for 5 minutes each in PBS (pH 7.6), rinsed in distilled water and air-dried. Coverslips were mounted with 90% glycerol. Slides were examined by immunofluorescence microscopy and fluorescent foci were counted. A locus with 2 or more contiguous fluorescing cells was considered a focus.

Cell cultures

Two cell culture systems were used in this research.

1. Virus isolation Cells used for virus isolation were embryonic mule skin (EMS) cells, developed by Dr. W. A. Malmquist at the National Animal Disease Center, Ames, IA. Cultures were grown in Eagle's MEM supplemented with 10% FCS (fetal calf serum) and 100 units penicillin G potassium, 100 µg streptomycin sulfate and 100 µg kanamycin sulfate/ml. Maintenance medium was identical to growth medium except the FCS was reduced to 2%. Cultures were grown in Falcon flasks and glass chamber slides in a humidified 5% CO_2 atmosphere at 37° C.

2. Virus multiplication Embryonic equine kidney (EEK) cells were provided by the National Veterinary Services Laboratory, Ames, IA. They were grown in Eagle's MEM supplemented with 15% FCS, 75 µg/ml gentamycin (Gentocin^R Schering) and 10% L glutamine. Maintenance medium was identical to growth medium except for the FCS which was reduced to 10%. Falcon flasks, Leighton tubes, and wells were incubated at 37° C with 5% CO_2.

Serum neutralization tests

Control virus used for the test was a second passage virus on EEK cell culture with a titer of 1.5×10^5 FFU/ml. Inactivated test sera, positive and negative controls were diluted 1:2 in tissue culture medium with doubling dilution to 1:512. Control virus was diluted 1:100 in MEM medium containing 10% guinea pig serum as a source of complement (36,37) and 1 ml virus dilution was added to an equal volume of each test serum dilution. Monolayers of EEK cell culture grown on 16 mm diameter wells (Tissue Culture Cluster, Costar) were inoculated with 0.4 ml serum-virus mixture to 4 wells per dilution. After 1 hr incubation at 37° C, each well was overlayed with 1 ml agar medium and incubated for 72 hrs at 37° C. After cell fixation in 10% buffered formalin for 30 minutes, the agar plugs were removed and the culture was stained with Gram's crystal violet for 15 minutes.

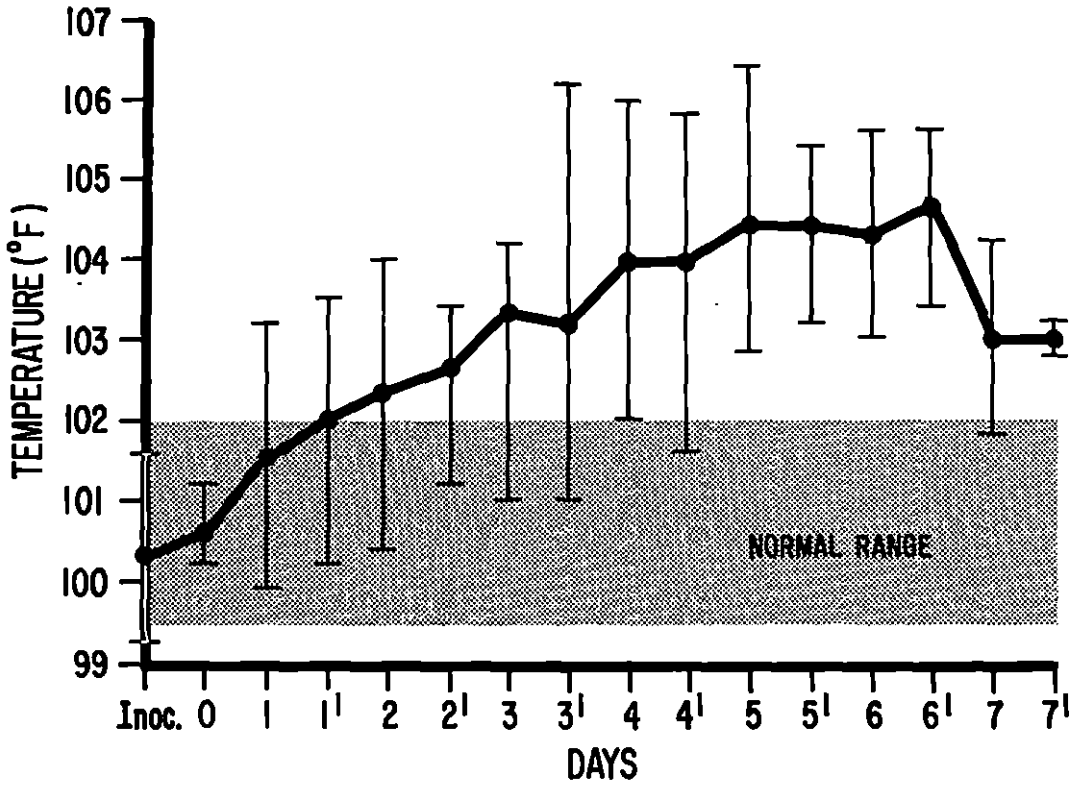
The serum neutralization titer was determined as the highest dilution of serum giving a 50% reduction in plaque number.

RESULTS

Clinical data

The control horse (#8) remained clinically normal during the whole study. Anorexia and lethargy appeared within 24 hrs in 6 of the 7 horses inoculated with EVA virus. Respiratory signs included serous bilateral nasal discharge followed by lacrimation. Two animals had increased respiratory rates and dyspnea; they both died from lung edema within five days after inoculation. Progressive prostration was observed in the other horses. In the terminal phase of the disease, the horses would not stand and became insensitive to external stimulations. The animals had various signs of colic, and remained with their mouths in the water, moved their lips, and did not drink. No sign of icterus or leg edema was observed. Among the 7 horses, 2 died. A third horse did not have clinical signs and was killed at day 9. Three were euthanized in the agonal phase of the disease within 5 days after inoculation. The last mare recovered, aborted 9 days postinfection and was necropsied 24 hrs later. Fever was an early sign and was present in all infected animals including the horse which had no other clinical manifestation of disease (mare #1). Rectal temperatures over 101.5^{o} F were considered abnormal. Fever started 24 hrs postinoculation in 5 animals, 2 days postinoculation in one and 4 days postinoculation in mare 1. Temperatures increased between the second and the sixth day postinfection with an average of about 104.5^{o} F on days 5 and 6. Figure 2 indicates the range of temperatures.

Figure 2. Average rectal temperatures during EVA. Morning (1,2,3,...), afternoon (1',2',3',...). Vertical lines represent the range


107
106
105
104
103
102
101
100
99
TEMPERATURE (°F)
NORMAL RANGE
Inoc. 0 1 1' 2 2' 3 3' 4 4' 5 5' 6 6' 7 7'
DAYS

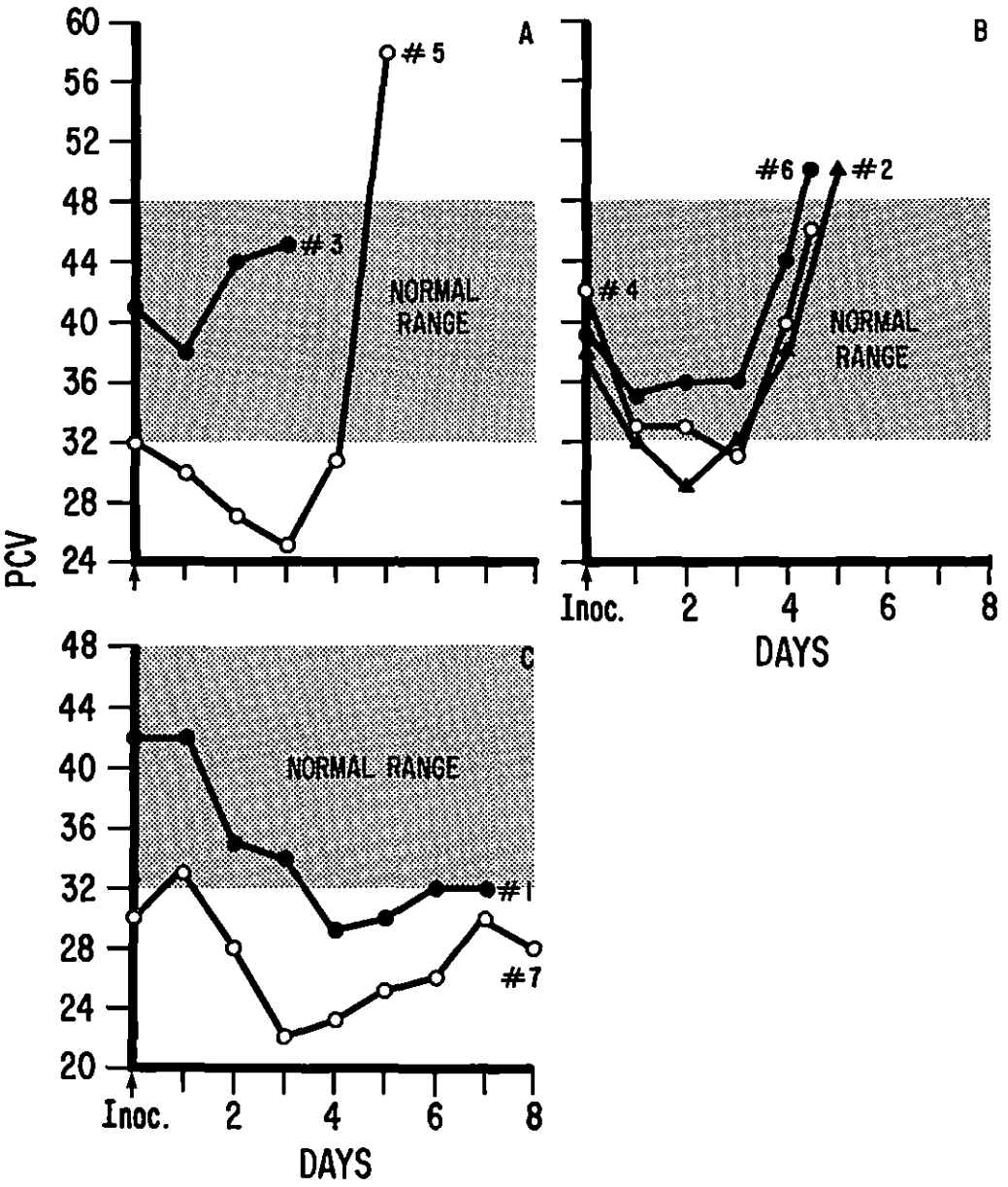
Packed cell volume (PCV) is presented for each animal, from inoculation to death. The horses have been divided in 3 groups corresponding to the evolution of the clinical signs (Figure 3). Initial values of PCV were between 30 and 42%, 6 of the 7 horses being within normal limits of 32 to 48% (17). An initial drop in PCV occurred between day 1 and day 3 postinfection in animals of group I and II, at day 3 and day 5 respectively for the horses of group III. Four horses had a PCV below normal limits for a transient time, mare 7, which aborted, remained below normal for 7 days until euthanasia. Rapid increase of PCV occurred on day 3 p.i. in the three horses of group II and in one horse of group I. Three animals were over upper normal limits at the time of death or euthanasia.

Red blood cell (RBC) counts were within the normal range in 5 of 7 horses at the time of inoculation (Figure 4). Horses 2, 3, 4, and 6 had the lowest count 2 days after inoculation, horses 5 and 7 on day 3, horse 1 on day 6. Mare 7 remained below normal during the whole trial. Mare 5 remained below normal from inoculation to day 4 and was within normal values before death.

Platelets were within normal limits of 100-600 $10^3/mm^3$ (17) except for those of mares 4 and 6. Drops of 34 to 62% in platelet counts were recorded in all but one horse between day 3 and day 5 postinfection. Horses 1 and 7 survived. In the 5 horses which did not survive the acute phase of the disease, the lowest values were found at the time of

Figure 3. Packed cell volume (PCV)

A - Group I. Horses found dead
B - Group II. Horses killed *in extremis*
C - Group III. Surviving horses


A
B
C
PCV
DAYS
Inoc.
NORMAL RANGE
#1
#2
#3
#4
#5
#6
#7
60
56
52
48
44
40
36
32
28
24
20
2
4
6
8

Horse	Day of Inoc.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
1	7.2	6.4	6.3	6.6	5.7	5.7	5.4*		
2	6.5	-	4.8*	5.9	6.2	8.2			
3	7.2	7.0	6.9*	7.7					
4	6.9	5.9	5.7*	8.4	6.5				
5	5.3	5.1	4.7	4.1*	5.2	8.6			
6	6.3	6.5	6.2*	6.3	7.3	8.3			
7	5.2	5.7	5.7	4.4*	4.7	4.6	4.6	5.5	5.4

____ indicates the figures below normal

* indicates the lowest value for each horse

Figure 4. Red blood cell counts (in $10^6/mm^3$)

euthanasia "in extremis" (#2, 4, 6) or just before natural death (#3, 5). These results are illustrated on Figure 5.

At inoculation, white blood cell counts were between 8.5 and 13.1x 10^3 cells per mm^3 blood, with an average of $9.8x10^3$ (Figure 6). On day 2, the average was $6.6x10^3$ cells/mm^3. All animals were leukopenic or within low normal range. Three days after inoculation, the lowest value was reached for the group with an average of $6.2x10^3$. The figure recorded on day 6 concerned only the 2 horses which survived the acute phase of the disease. The minimal WBC count for each horse is given in Figure 7 with the day it occurred and the eventual evolution of the disease in this animal.

Progesterone levels were not significantly different before or after inoculation in the mares which did not abort. Figure 8 illustrates the variations of progesterone levels in plasma of mare 7, which aborted 8 days after inoculation. Before inoculation, progesterone levels varied between 9.6 and 14.4 ng/ml. The day of abortion the level was 3 ng/ml.

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and its metabolite 13,14-dihydro-15-ketoprostaglandin $F_2\alpha$ (13,14-OH-15ketoPGF$_2\alpha$) were not significantly affected by infection and disease.

Lactic dehydrogenase (LDH), sorbitol dehydrogenase (SDH) and ornithine carbamyl transferase (OCT) amounts in serum are given in Figure 9.

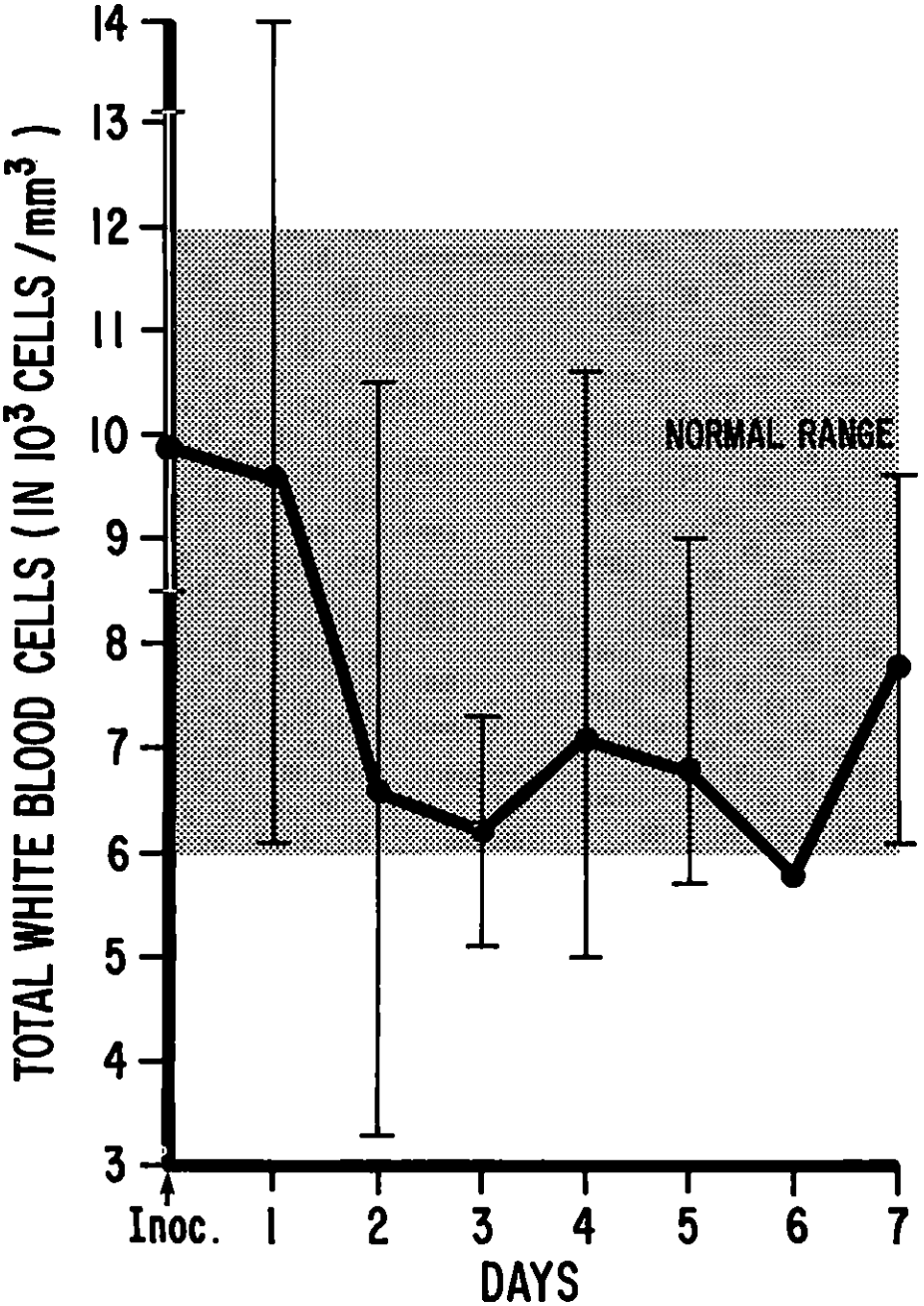
Lactic acid levels in plasma varied from 1.7 to 7.6 in mMoles/l before infection. No significant changes were found in the circulating blood during the whole observation period.

Horse	Inoculation (1)	Minimal value (2)	Day (3)	Drop (in %) (4)
1	234	211	4	34
2	275	124	5	55
3	218	214	3	2
4	155	79	4	50
5	211	103	5	51
6	235	89	4	62
7	244	115	5	53

1. Averages before inoculation
2. Absolute minimum value
3. Day where minimum value was reached
4. Percentage decrease compared to preinoculation levels

Figure 5. Platelet counts (in $10^3/mm^3$)

Figure 6. Total white blood cell averages. The vertical lines give the range


TOTAL WHITE BLOOD CELLS (IN 10^3 CELLS / mm^3)
14
13
12
11
10
9
8
7
6
5
4
3
NORMAL RANGE
Inoc.
1
2
3
4
5
6
7
DAYS

Horse No.	Minimal WBC Count	Day	Evolution
1	5.8	5/6	S
2	4.1	2	E day 5
3	6.3	3	† day 4
4	5.2	3	E day 4
5	3.3	2	† day 5
6	4.1	2	E day 4
7	5.7	5	S

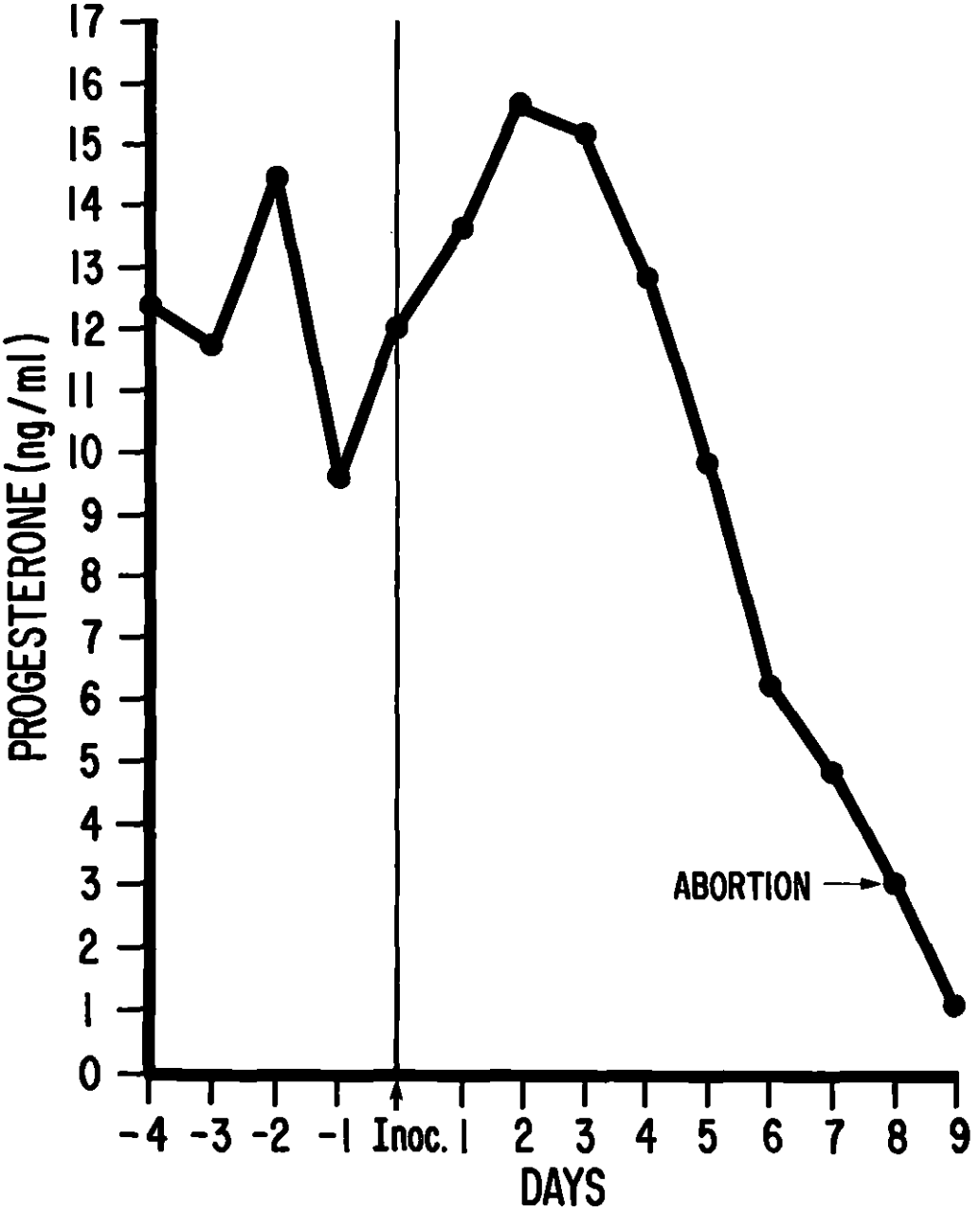
S = survival

E = euthanized "in extremis"

† = found dead

Figure 7. WBC counts, minimal values

Figure 8. Progesterone levels in serum (in ng/ml), mare 7 .


PROGESTERONE (ng/ml)
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ABORTION
-4 -3 -2 -1 Inoc. 1 2 3 4 5 6 7 8 9
DAYS

Horse No.	Before Inoculation			After Inoculation		
	LDH	SDH	OCT	LDH	SDH	OCT
1	-	-	-	659	3.6	0.76
2	690	6.3	1.35	629	8.0	1.11
3	631	7.1	0.80	603	9.3	1.93
4	660	6.7	3.29	571	6.7	1.81
5	797	4.4	1.03	752	6.5	0.87
6	586	3.0	1.04	565	3.6	1.19
7	505	4.3	3.57	728	4.0	3.01

LDH = lactic dehydrogenase

SDH = sorbitol dehydrogenase

OCT = ornithine carbamyl transferase

Averages before inoculation (1st number) and after (2nd number) are given for each animal.

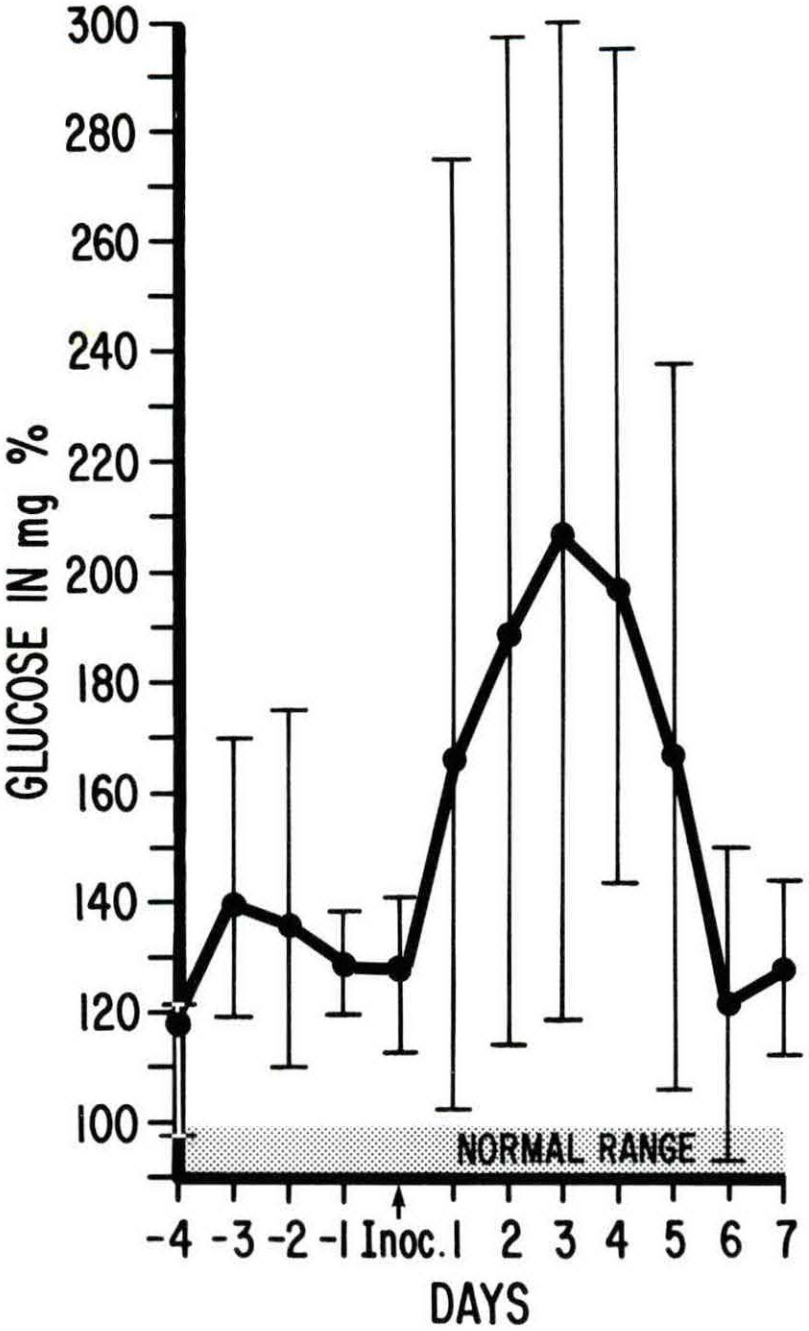
Figure 9. Blood enzymes (in I.U.)

Blood urea nitrogen (BUN) levels were normal in infected animals. Serum glucose increased in the 6 horses with clinical signs. Highest glucose amounts occurred at day 2 p.i. for one horse (#6), at day 3 for 3 horses (#2, 3, and 7), and at day 4 for 2 horses (#4 and 5). Maximum values were reached just before death in 3 animals (#3, 4, and 5). Figure 10 gives the average and range of glucose concentration before and after inoculation. Variations of serum electrolytes (Na, K, Ca, Mg, Cl, and P) from the normal range were not wider at any time after inoculation than before. Creatinine and gamma glutamyl transpeptidase amounts in plasma remained within normal range during the whole observation period.

Gross lesions

The results of infection are tabulated in Figure 11. In mare 2, serosa and fat tissue appeared edematous in the lumbar area, around kidneys, and along the root of the anterior mesenteric artery. Mare 3 was found dead. Lungs were enlarged, heavy, congested, and uniformly edematous. Foamy mucus filled bronchi and trachea. Subcutis and subcutaneous fat were infiltrated by serous fluid. Fetal membranes containing about 2 liters of clear fluid emerged from the vagina, lying on the perineum. Mare 5 was found dead with pulmonary lesions comparable to mare 3. No gross lesions were found in mare 6. In mare 7, killed 24 hrs after abortion, a diffuse congested appearance of the uterine mucosa was the only lesion found. No gross lesion and no evidence of autolysis were seen in the aborted fetus.

Figure 10. Glucose levels in serum before and after inoculation


300
280
260
240
220
200
180
160
140
120
100
GLUCOSE IN mg %
NORMAL RANGE
-4 -3 -2 -1 Inoc. 1 2 3 4 5 6 7
DAYS

Mare No.	Pregnancy (est. in days)	Course of disease	Fetus at date of necr.
1	>300	fever	alive
2	160-180	acute-fatal	alive
3	160-180	acute-fatal	dead
5	160-180	acute-fatal	dead
6	>300	acute-fatal	alive
7	>300	abortion	alive
8 Control	>300	no clinical sign	alive

Figure 11. Evolution of the disease in mares and fetuses

Microscopic lesions

Uterus of the mare is lined by a simple columnar epithelium. A loose connective tissue, highly vascular, propria-submucosa separates the epithelium from the myometrium. Myometrium consists of an inner circular and outer longitudinal smooth muscle layer separated by a vascular zone (12).

Equine placenta is diffuse, non deciduate, villous, and epitheliochorial. Around 100 days gestation, trophoblast and maternal epithelium form microcotyledons, tufts of chorionic villi invaginate in corresponding crypts of the endometrium. A thinning of maternal epithelium occurs as gestation progresses (38).

Ultrastructure of the placenta at 250-300 days gestation is characterized by large trophoblast cells packed with smooth endoplasmic reticulum. Lipid droplets and crystalline inclusions are occasionally present and electron dense bodies of various shapes, sometimes lamellated, are randomly distributed in the cytoplasm or aggregated near the nucleus.

The maternal epithelium is flattened. Nuclei appear irregular and condensed and the cytoplasm contains few organelles (38). Figure 12 illustrates the feto-maternal junction in the control mare.

Lesions of the genital tract Extensive endothelial and media lesions, typical of EVA, were found in blood vessels in ovaries of mares 4 and 5 (Figure 13).

In the uterus of all the pregnant mares, slight diffuse edema was present in the lamina propria in areas of placentation. Surface and

Figure 12. Fetal capillary (FC) closely apposed to a trophoblastic cell (T). Electron dense bodies and lipid vacuoles (L) in the cytoplasm. Note the intercullar channel between adjacent trophoblastic cells (arrow). Uterine epithelium (U) with smooth ER, basal rough ER, elongated mitrochondria and secondary lysosomes (Ly). Normal placenta. Mare 8

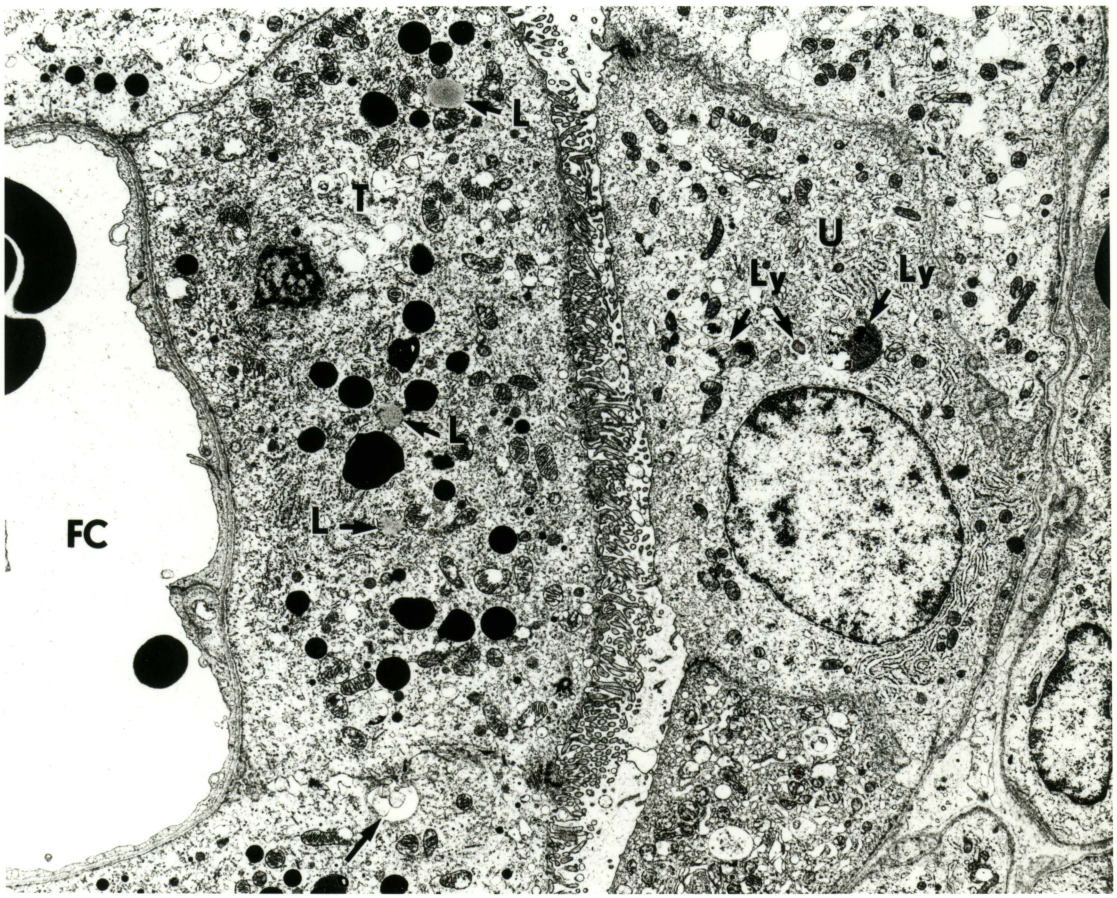
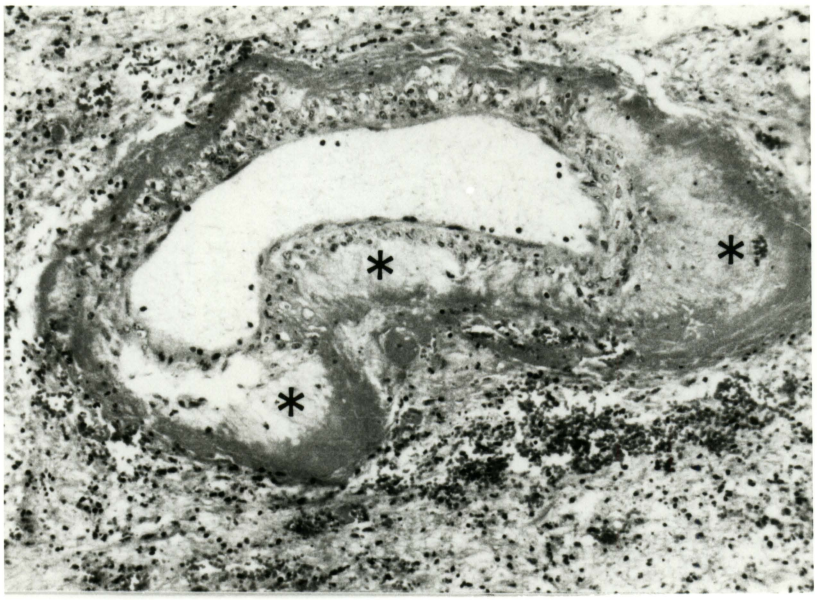
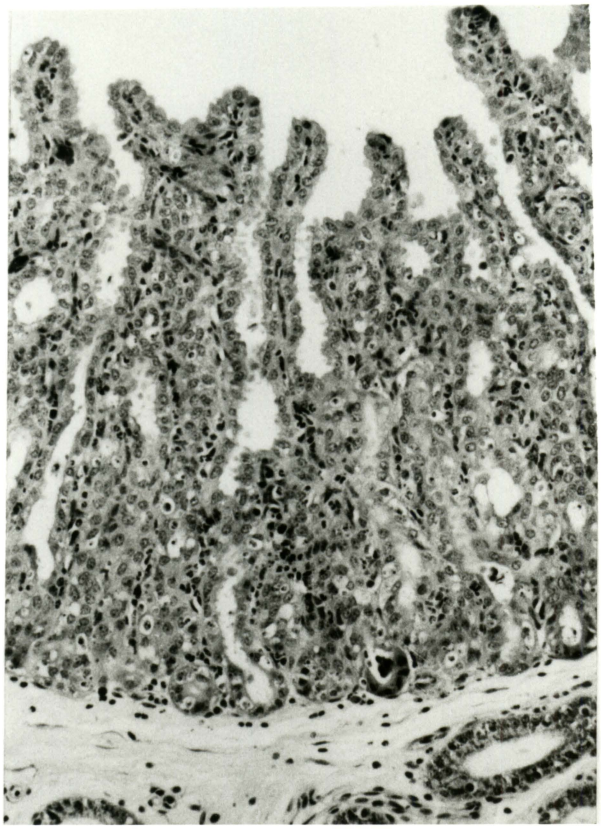

L
T
U
Ly
Ly
L
L
FC

Figure 13. Ovary. Extensive necrosis of the arterial wall with fibrin deposits (*). Perivascular edema and hemorrhage (lower right)

Figure 14. Endometrium. Diffuse infiltration of the propria by polymorphonuclear neutrophils (mare 7)



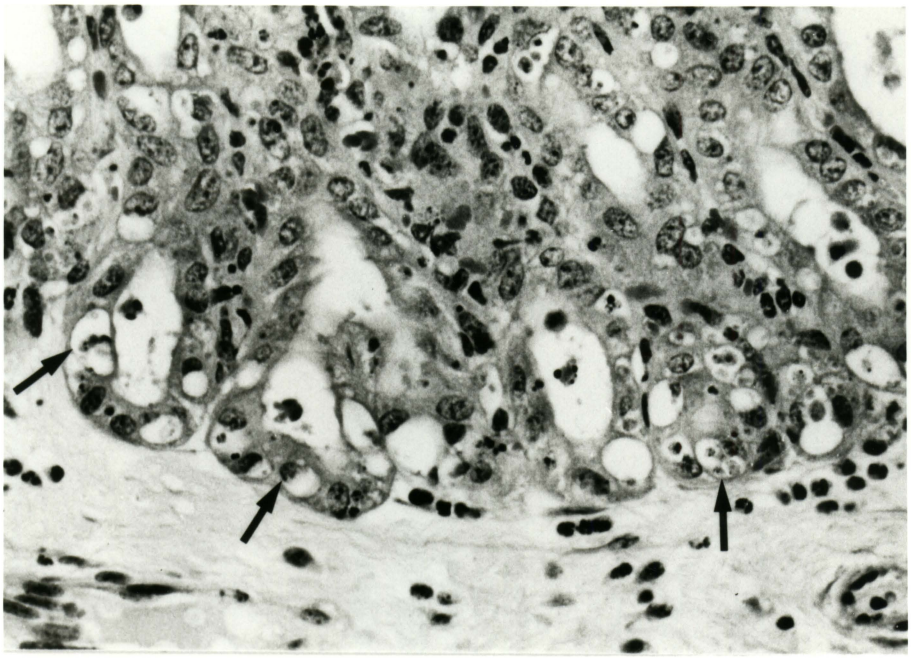


glandular epithelia, myometrium and blood vessel walls appeared normal in six animals and they are not described further.

Mare 7 had extensive endometrial and myometrial lesions. The superficial layer of the propria-submucosa, between the epithelial folds, was diffusely infiltrated with neutrophils (Figure 14). Epithelial cell vacuolation was observed. The percentage of vacuolated cells was estimated at about 10% of the whole surface epithelium. Vacuolated cells were found throughout the uterine mucosa, most vacuoles contained a clump of eosinophilic material and granular basophilic debris (Figure 15). The lumen between epithelial folds contained homogeneous eosinophilic material, necrotic cells, and, less often, polymorphonuclear neutrophils. Endometrial glands had similar lesions. Individual cell vacuolation was present in every gland examined (Figure 16). In many glands, the vacuoles were filled with small granules of green-brown pigment. Lumens of the glands contained eosinophilic material and, occasionally, necrotic cells. Vacuoles containing lipids were seen in the uterine epithelium stained with oil red O. In contrast, large lipid globules were consistently present in vacuolated cells of endometrial glands. Fibrin was not detected in endometrial or myometrial blood vessels. Myometrium was extensively affected in both inner circular and outer longitudinal muscle layers. The internal part of the inner layer was most severely damaged. Foci of inflammatory cells were found in most muscle bundles of the internal muscular layer. Adjacent connective tissue was spared (Figure 17). Inflammatory foci

Figure 15. Endometrium. Vacuolation of epithelial cells. Note debris in the vacuoles (arrows). Mare 7

Figure 16. Endometrial glands. Cell vacuolation. Mare 7



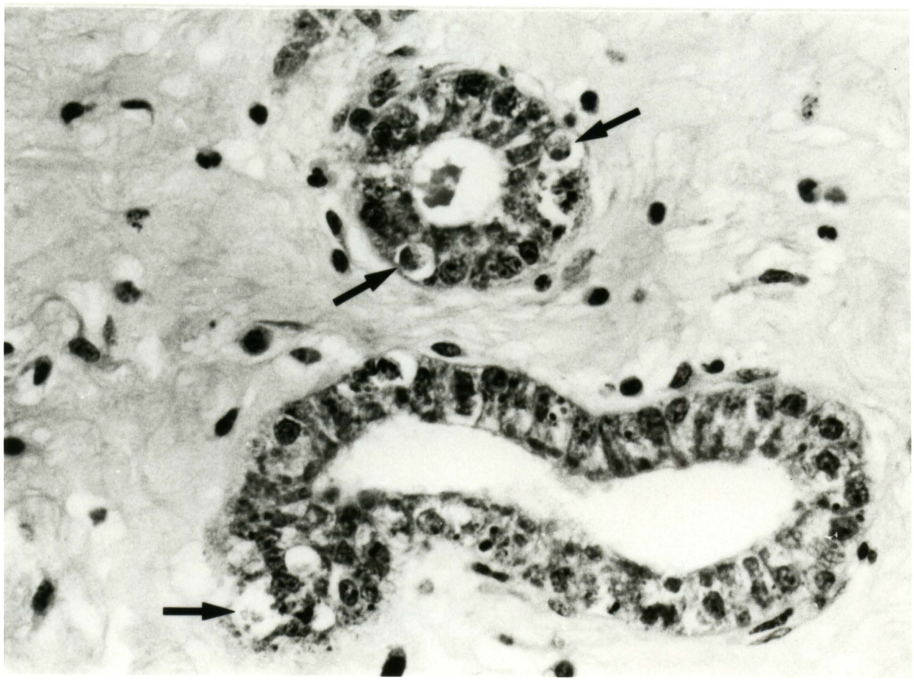
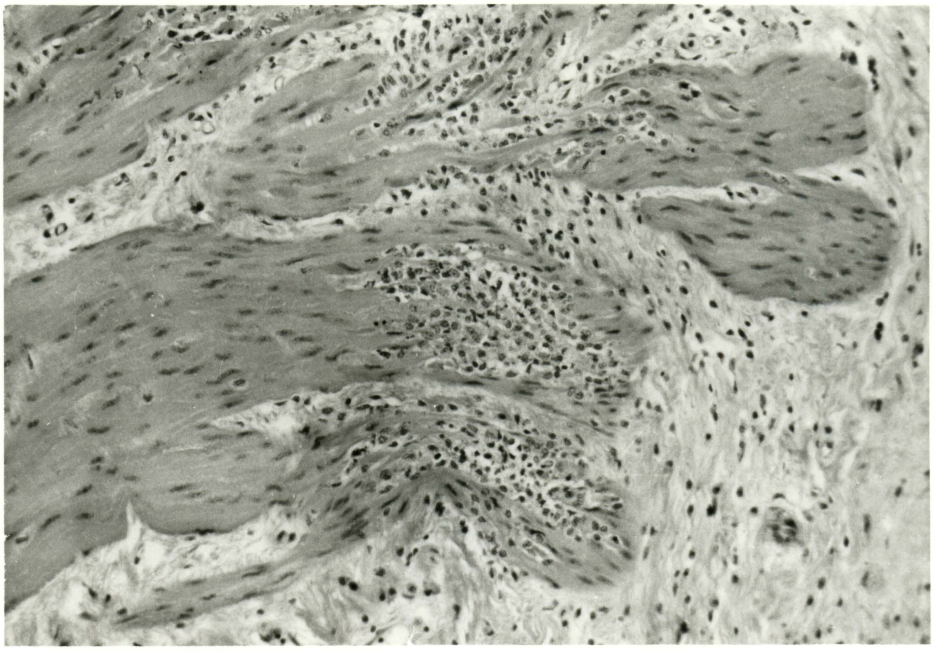
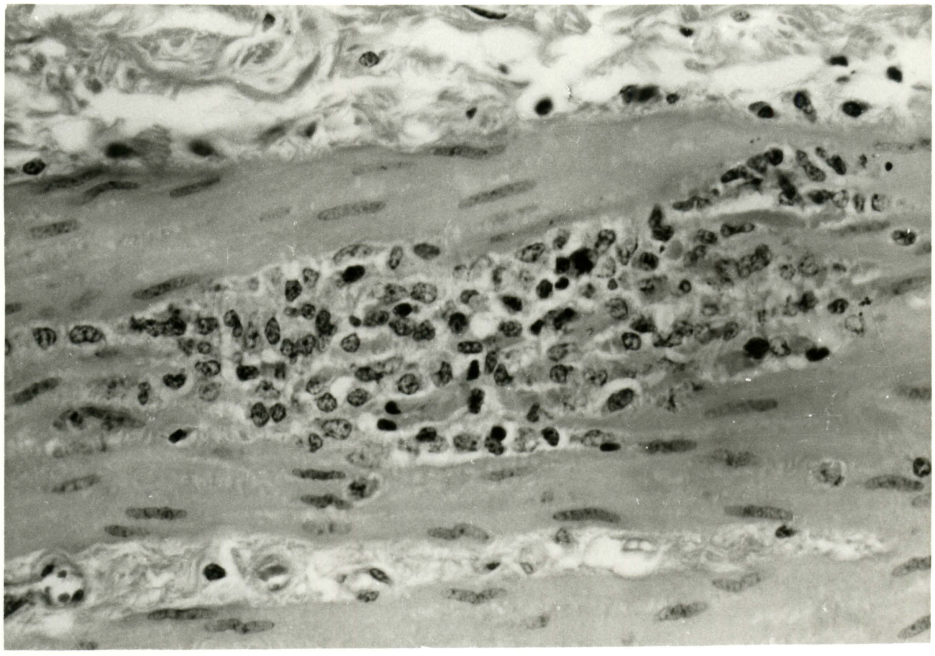


Figure 17. Smooth muscle necrosis and focal histiolymphocytic myometritis. Inner layer of myometrium. Mare 7

Figure 18. Myometrium. Foci of necrotic muscle containing macrophages and lymphocytes. Mare 7





contained macrophages and lymphocytes. Muscle fibers appeared necrotic in these areas with a loose network of fibrillar material and eosinophilic, dense clumps of fibrinoid necrosis (Figure 18). Similar lesions were present in all samples examined. No blood vessel alteration was found.

On electron microscopy, placenta appeared similar in mares 2, 4, and 6, except for the differences due to the stage of pregnancy. They will be described together. Morphology of mare 2 uterine epithelium and trophoblast layer is illustrated in Figure 19. In trophoblast, elongated mitochondria and dictyosomes of the Golgi complex were seen in various locations of the cytoplasm (Figure 20). Phagolysosomes, some of them containing a lamellar material, were occasionally found. Short lamellae and tiny vesicles of smooth endoplasmic reticulum filled the cytoplasm between the other organelles. Lateral cell membranes formed indentations with the neighbor cells. Junctional complexes formed of a superficial tight junction, an intermediate junction, and a desmosome were located in the upper part of the lateral wall. The cell surface had microvilli attached to corresponding structures in the maternal epithelium. The most external layer of cytoplasm contained numerous pinocytotic vesicles.

The uterine part of the placenta was composed of flat, irregular, epithelial cells with microvilli which interdigitated with the trophoblast layer. Cells laid on a thin, regular, basal lamina. Nuclei were usually finely granular with irregular borders. Small, variously shaped mitochondria were evenly distributed in the cytoplasm, and abundant cytocavitary network filled the cytoplasmic matrix. Intense cell

Figure 19. Swollen uterine epithelium (U). Trophoblast (T). Basal lamina (BL). Note the thin layer of connective tissue between capillary (C) and epithelium. Placenta, 160-180 d. gestation. Mare 2

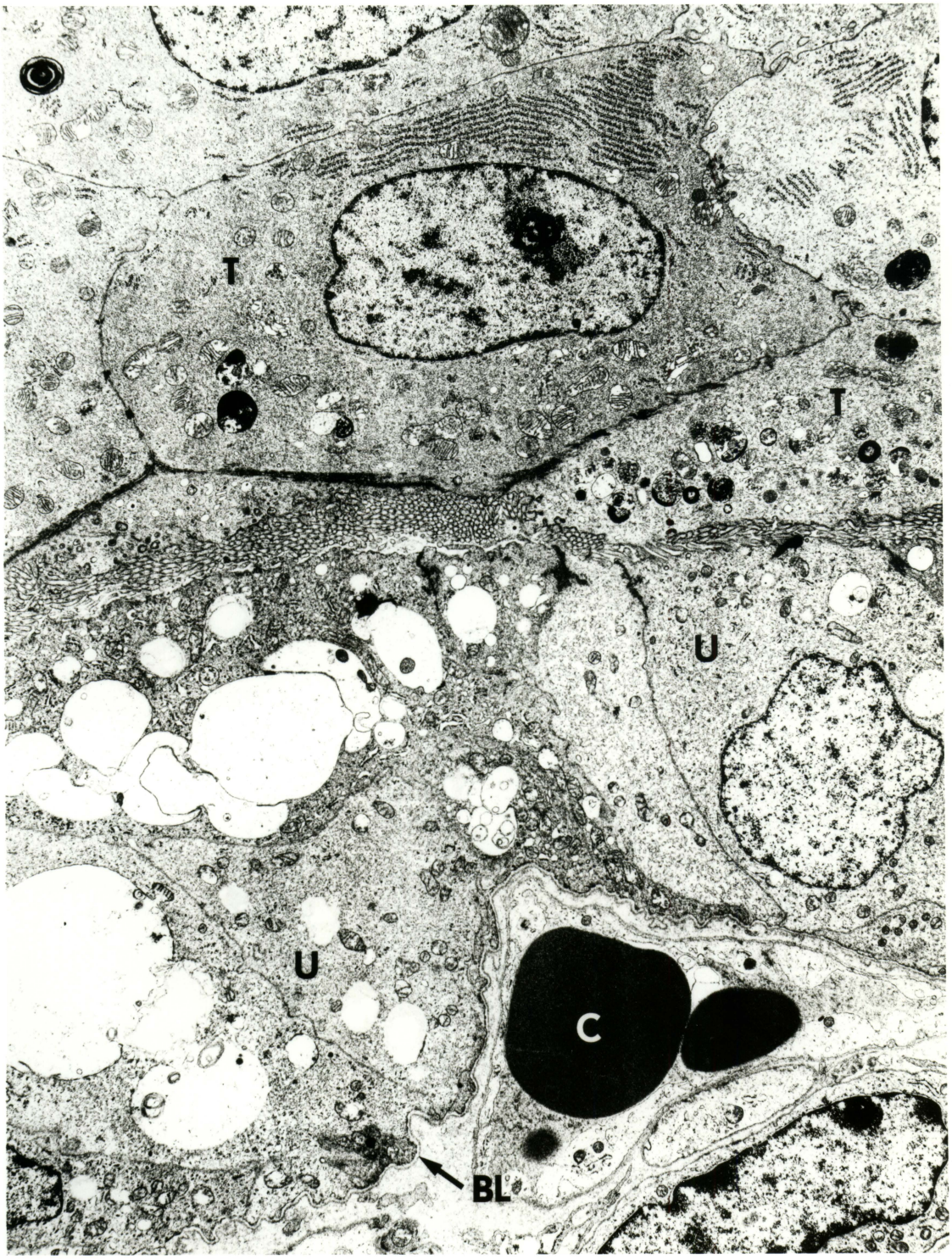
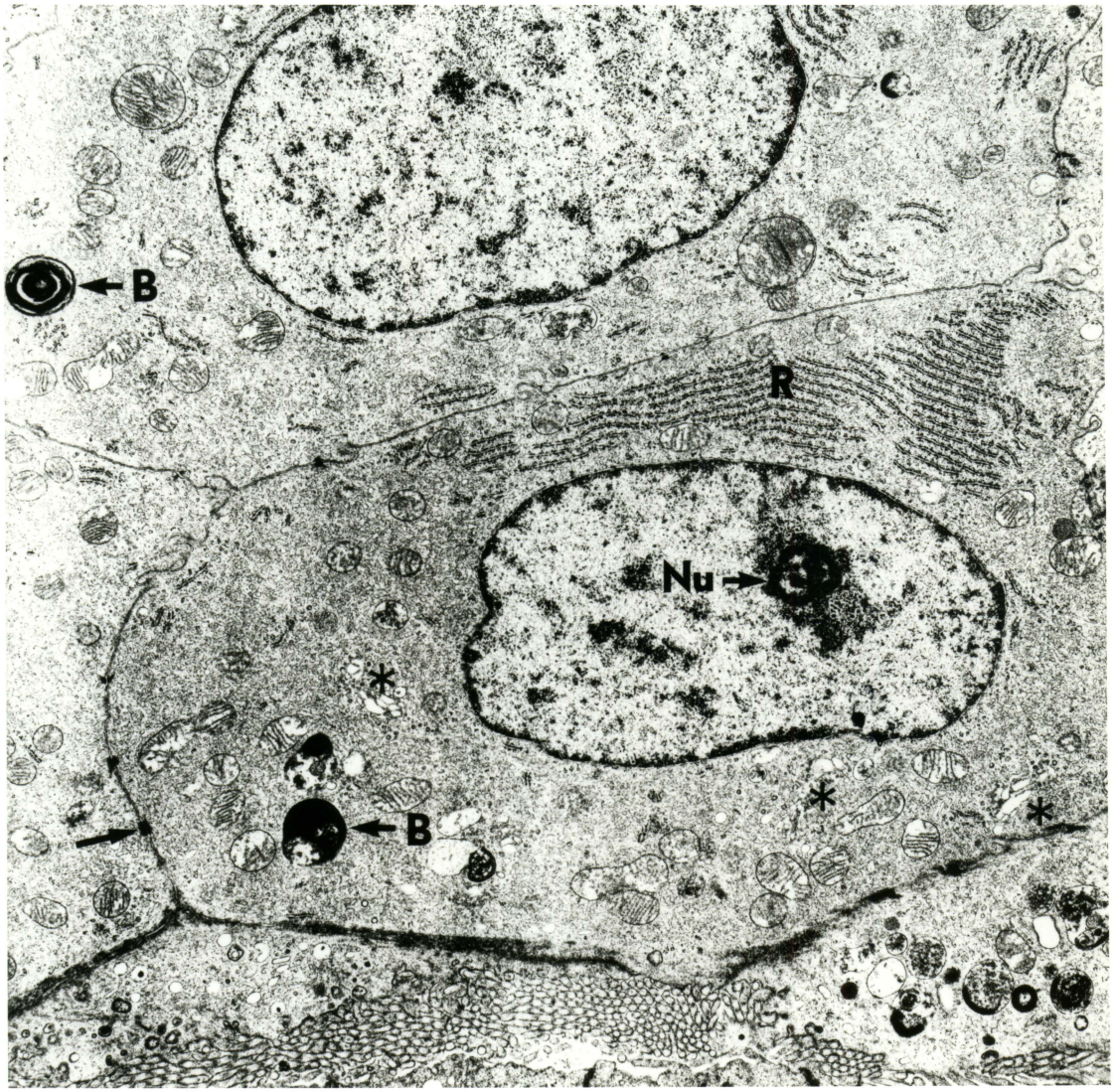

T
T
U
U
C
BL

Figure 20. Trophoblast. Oval granular nucleus with prominent nucleolus (Nu). Arrays of rough endoplasmic reticulum (R). Phagolysosomes (B) and dictyosomes of the Golgi complex (*). Note the abundant smooth endoplasmic reticulum and the junctional complex (arrow). Placenta. Mare 2


B
R
Nu
B

swelling was observed in uterine epithelium. Large residual bodies, solitary or in clusters were observed in all the cells examined. They were usually empty or contained small amounts of a loose granular material. Some were irregularly filled with lipids. Myelin figures were commonly present inside, or associated with the organelle (Figure 21). In mare 1, the placental barrier appeared normal.

The endometrium of the mare killed 24 hrs after abortion (#7) had large residual bodies containing membranous material and necrotic debris (Figures 22 and 23). Large clumps of electron dense material, stacks and concentric arrays of membranes, vesicles of various size and shape, and finely granular substance were also found (Figures 24 and 25). The endoplasmic reticulum of the cells was dilated and fragmented. Most mitochondria were swollen with precipitates of granular material in the matrix. Endothelium of capillaries was swollen, with perinuclear space dilatation, cytocavitary network swelling, lipid globules, and cytoplasmic vacuoles containing debris (Figure 26).

The capillary basal lamina appeared intact; no evidence of fluid leakage in the perivascular space was observed. In the lumen, red blood cells and granular proteinaceous material were observed, without any evidence of fibrin or platelet aggregation. Macrophages and edema were present in the connective tissue between myofibers of the myometrium. In smooth muscle cells, dark foci of ribosome accumulation were found in the cytoplasm (Figure 27). Necrotic debris of muscle cells were also present in the inflammatory foci.

Figure 21. Maternal epithelium. Intense cell swelling. Microvilli (MV). Trophoblast (T). Terminal web (TW) containing pinocytotic vesicles. Large vacuoles (V) isolated or in clusters appear empty or granular. Note the lipid droplets, the myelin figures (arrows), and the irregular contours of the cells. Placenta. Mare 2

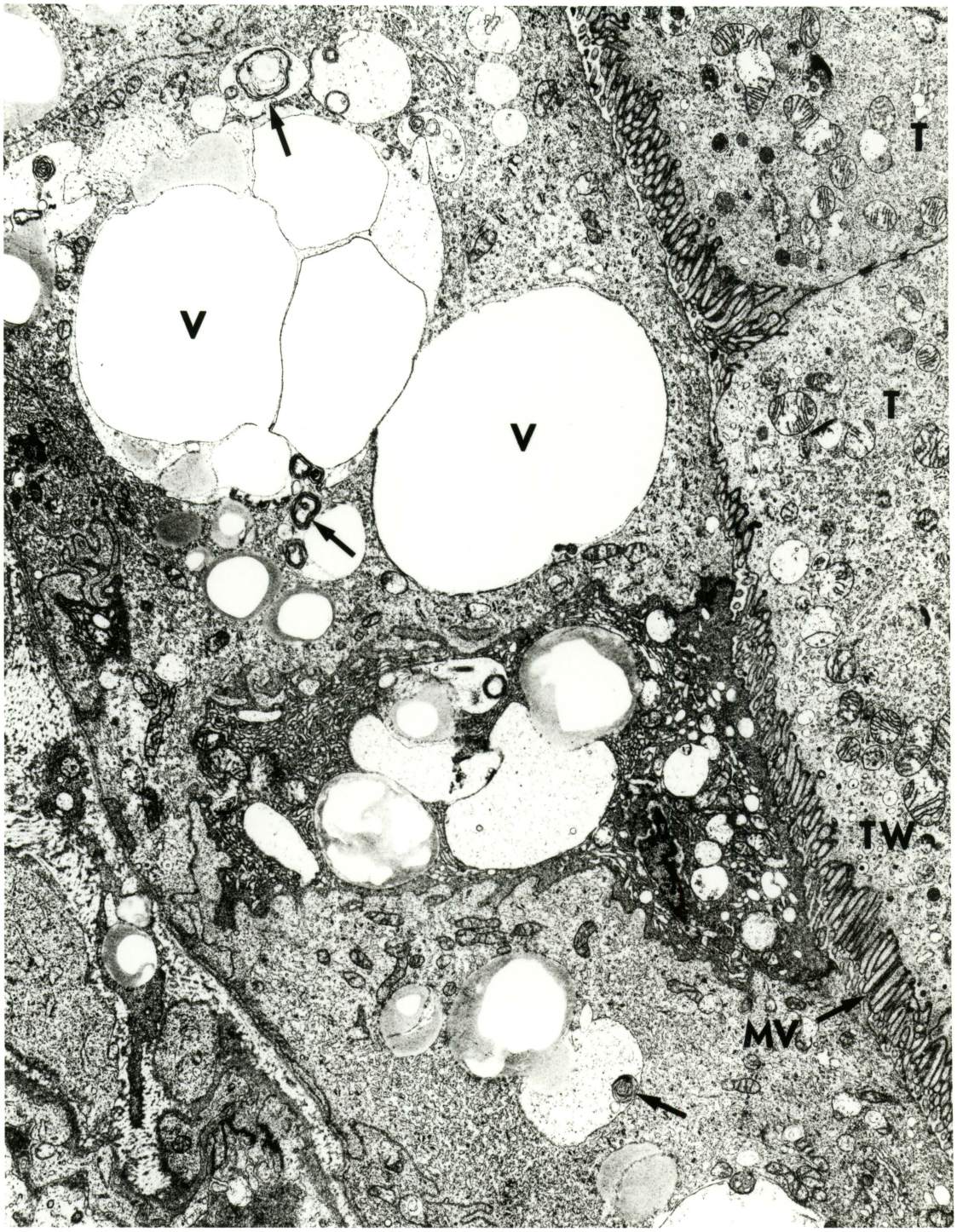

T
V
T
V
TW
MV

Figure 22. Endometrium. Residual bodies containing debris (*), dilated SER. Mare 7

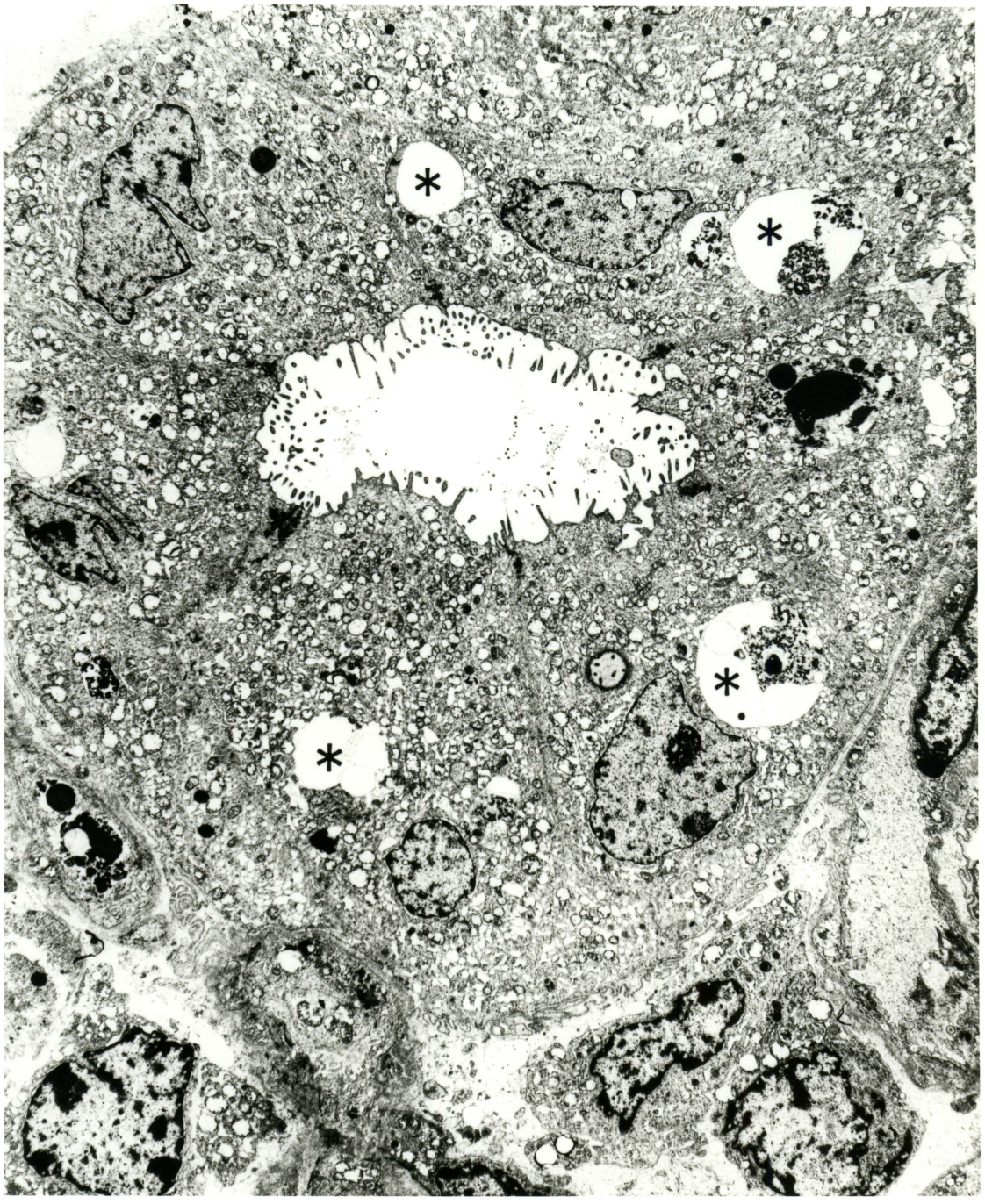


Figure 23. Cell swelling. Uterine epithelium. Smooth ER swelling and fragmentation. Large residual body containing membranous material and electron dense debris. Mare 7

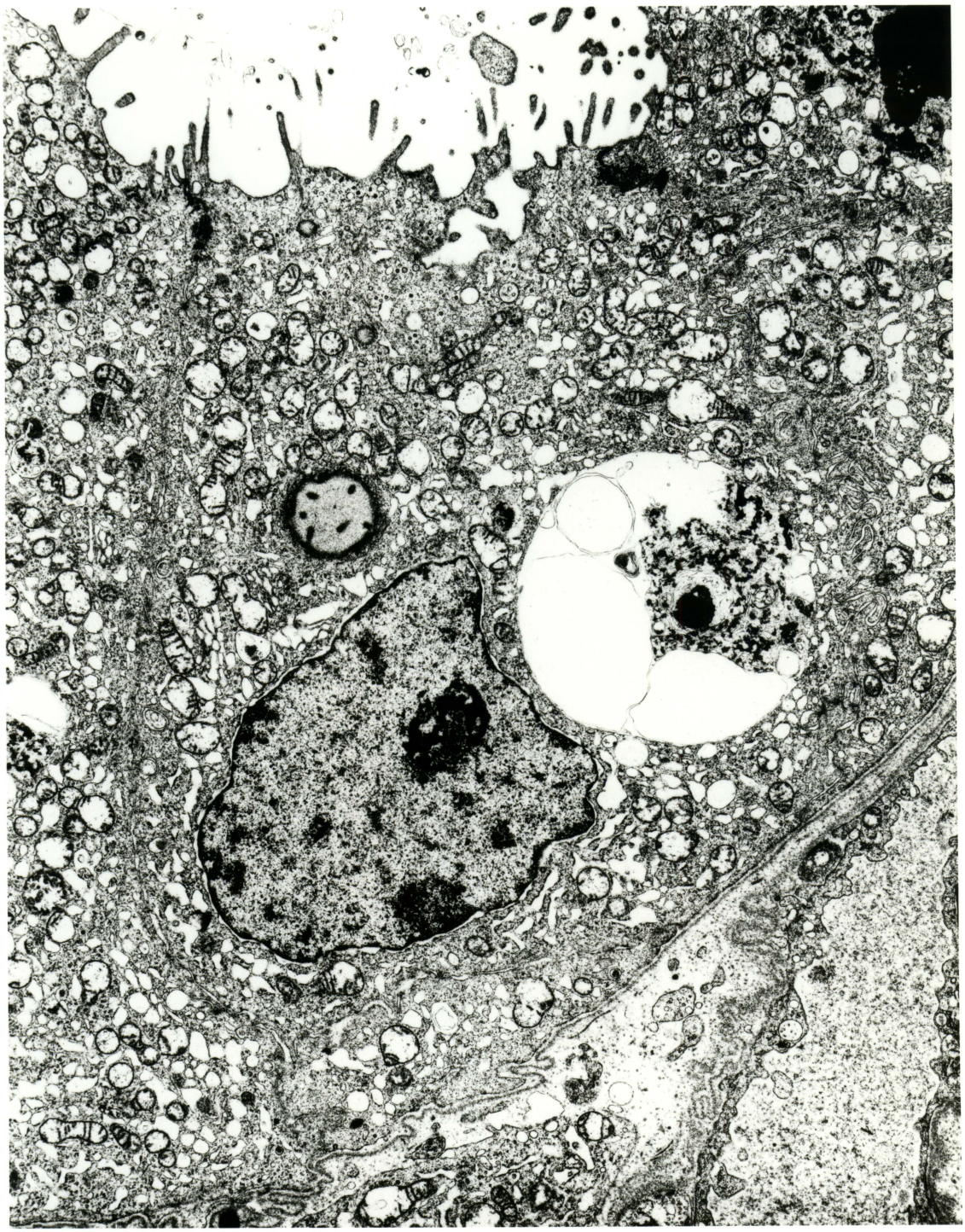


Figure 24. Various aspects of phagolysosomes and residual bodies. (1) Clear vacuole with delicate membranous network and necrotic debris. (2) Coarse electron dense granular material, multi-vesicular body. (3) Intracytoplasmic membrane aggregations. Endometrium. Mare 7

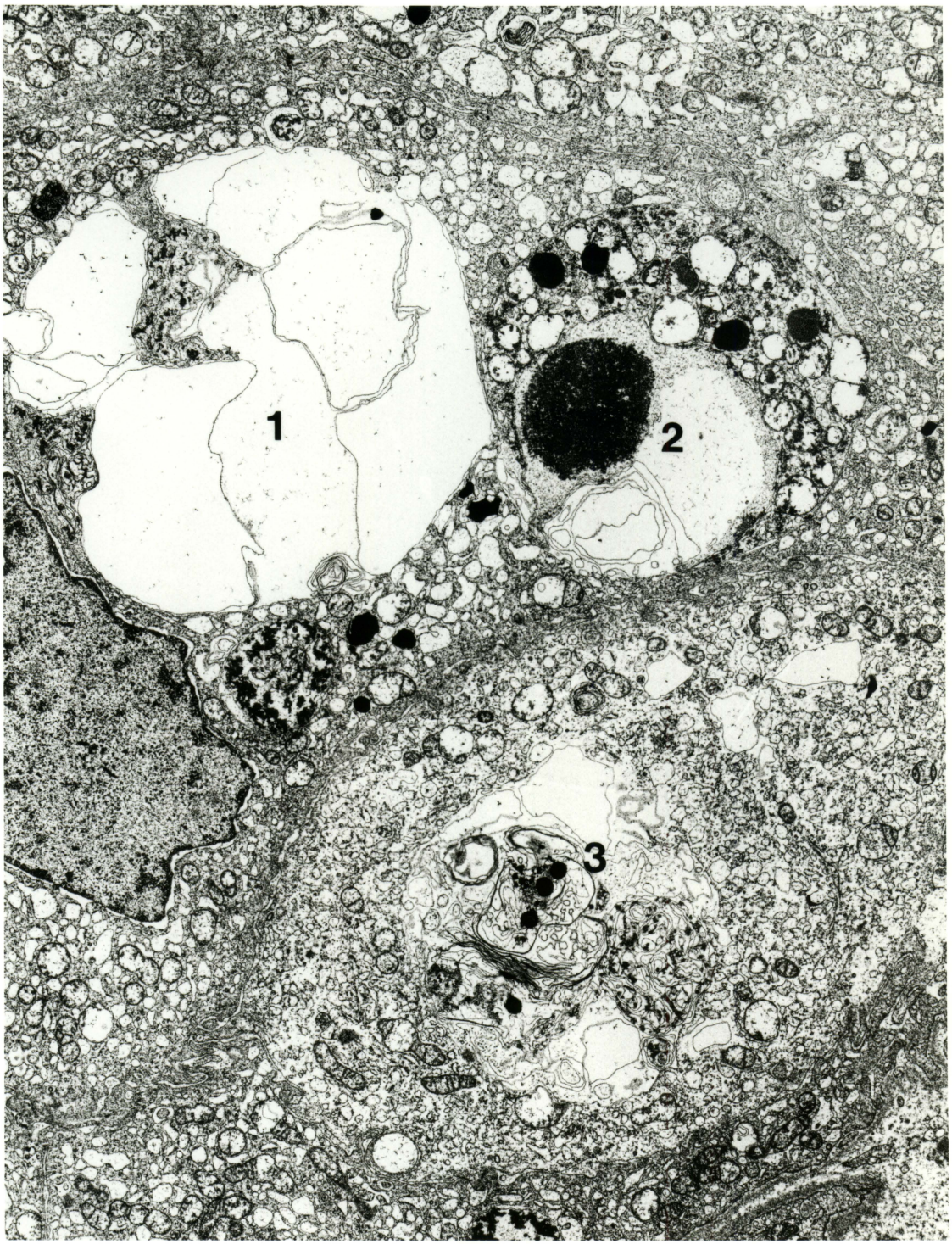

1
2
3

Figure 25. Concentric arrays of membranous material in a large residual body. Endometrium, mare 7

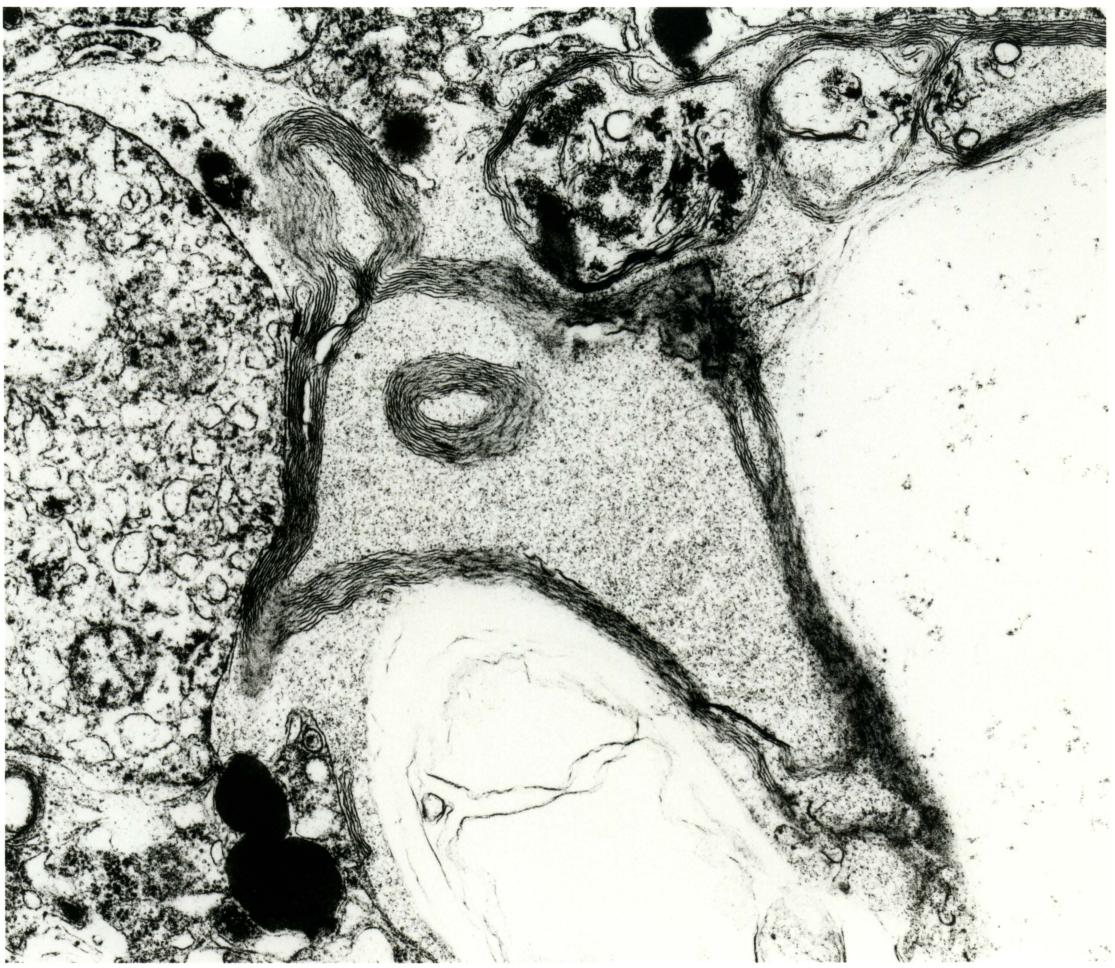


Figure 26. Capillary between epithelial cells. Endometrium. Swollen endothelium (E) with diacytotic vesicles. Lipid containing vacuoles (V). Phagolysosomes (arrows). Uterus, mare 7

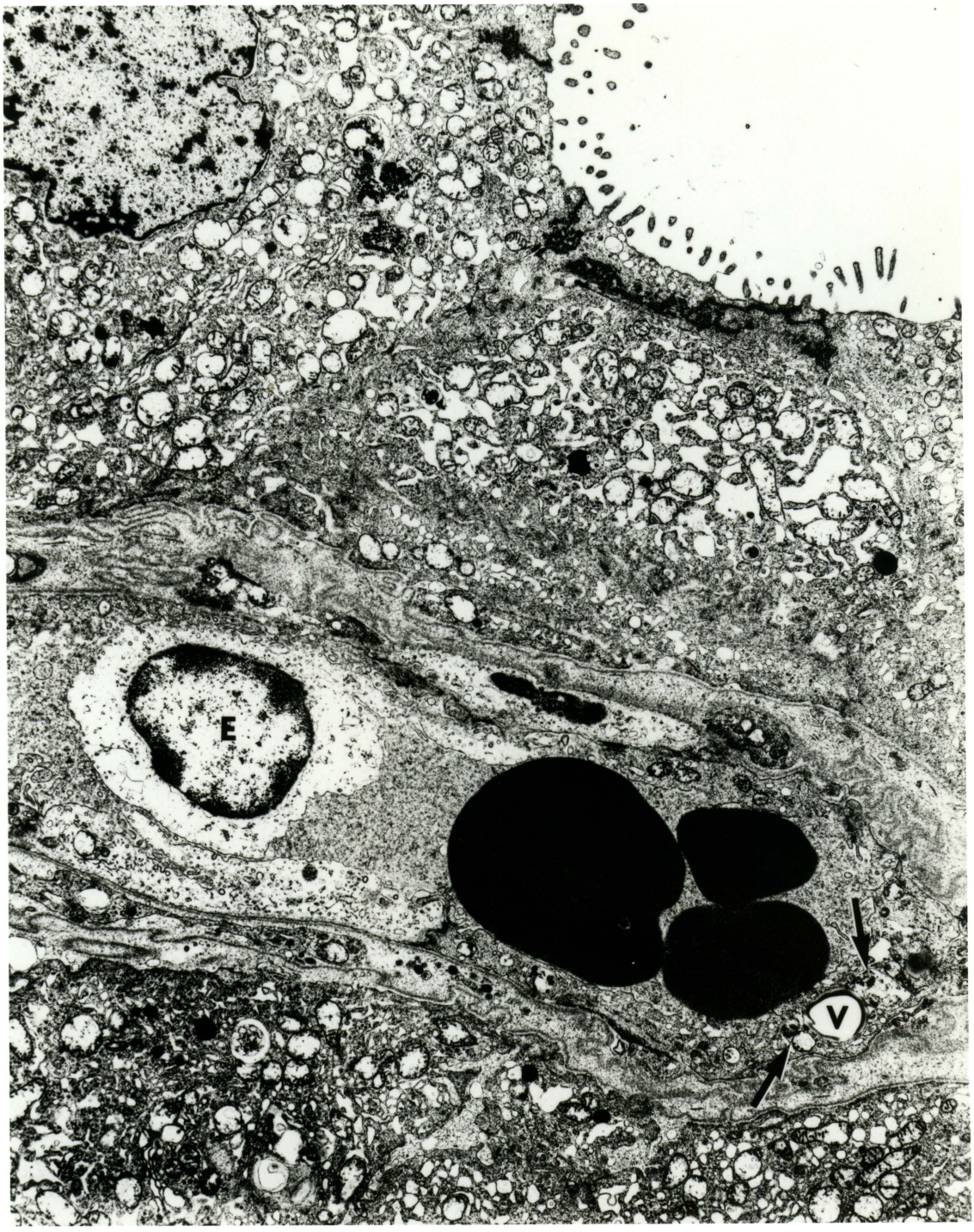
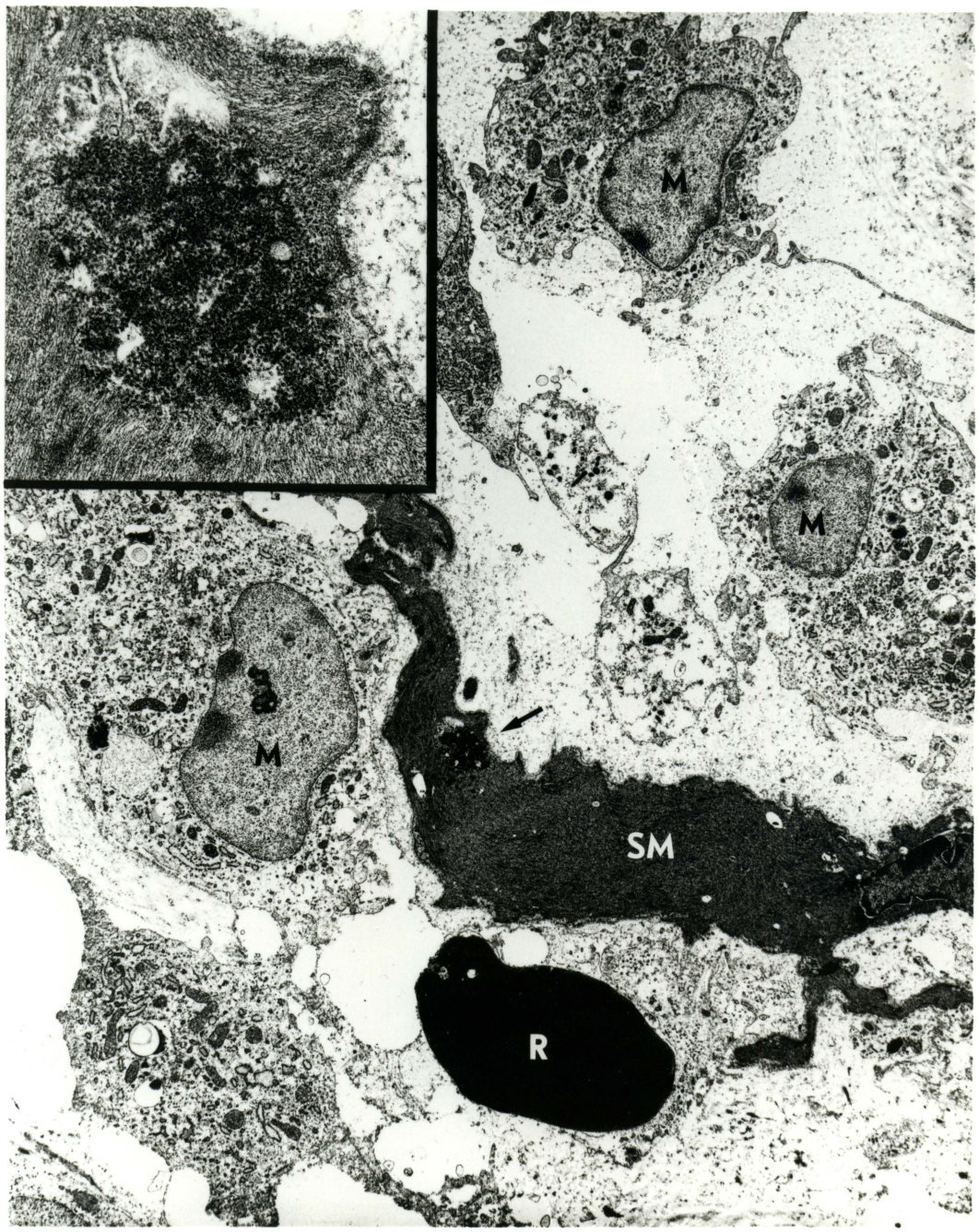

E
V

Figure 27. Smooth muscle cell (SM) with cluster of ribosomes along the cell membrane (arrow and insert). Macrophages (M). Red blood cell (R). Myometrium. Mare 7


M
M
M
SM
R

Lesions of the fetuses All but two samples of fetal and placental tissue examined by light microscopy appeared normal. Lesions were in umbilical cords of fetuses 6 and 7. In fetus 6, a pyogranuloma was present in the connective tissue, between the thick wall of an umbilical artery and the epithelium. It formed a bulge at the surface of the cord and was composed of a homogeneous, eosinophilic matrix with scattered small hemorrhages. A large number of foamy, large, macrophages surrounded the entire lesion. Microabscesses and fibroblasts were also present in the lesion (Figures 28 and 29).

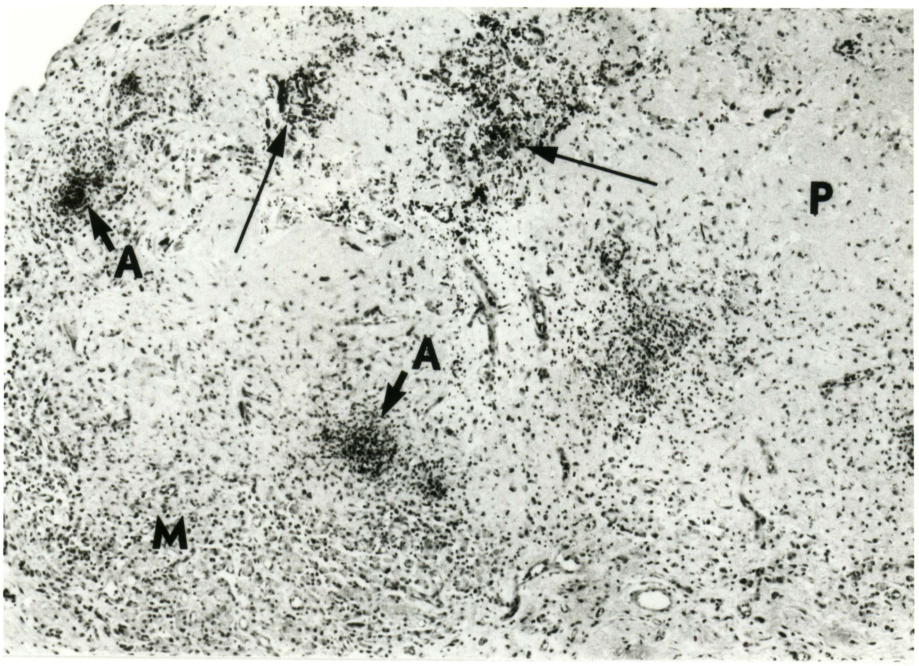
In fetus 7, chorionic epithelium and a layer of connective tissue were separated from the subjacent structures by a long slender cleft running parallel to the surface on about 25% of the section. The crevice contained homogeneous dense material, granules of a gold pigment and strips of solid eosinophilic substance resembling keratin. Accumulation of macrophages around the blood vessels and diffuse infiltration of the lesion by neutrophils were also observed.

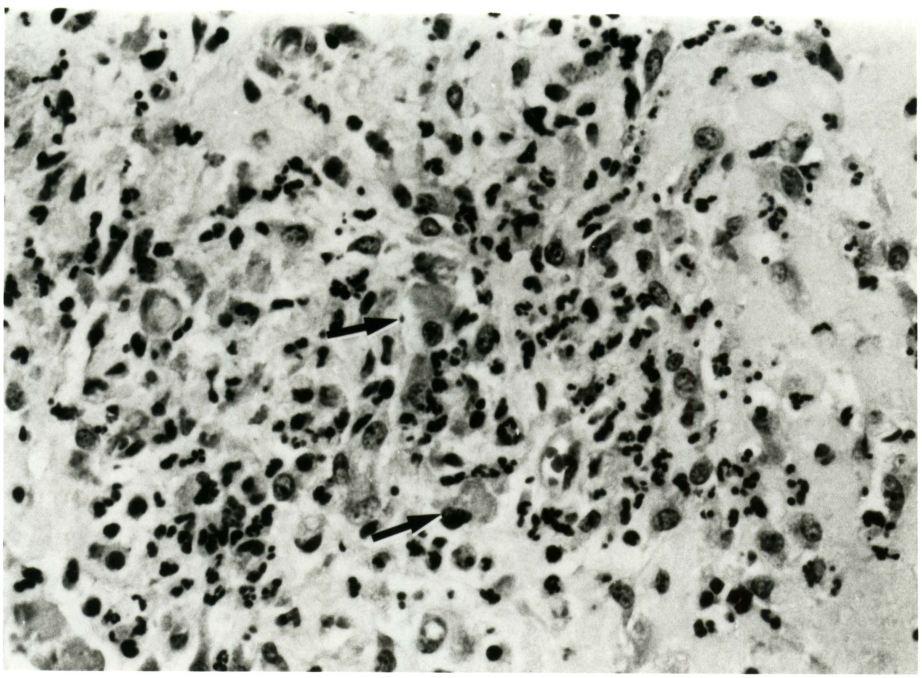
Lesions of the other organs Lesions varied only in intensity, therefore, an extensive description will not be given for each case. Figure 30 indicates which tissues were affected in each horse and gives an index specifying the intensity of the lesions. Mare 1 did not have any histologic evidence of EVA virus infection.

In lungs, edema was present around the bronchioles and associated blood vessels (Figure 31). Edema was also found in interlobular and subpleural spaces and diffusely distributed or surrounding small

Figure 28. Proteinaceous material (P) in the connective tissue. Infiltration by macrophages (M). Microabscesses (A) and hemorrhage (arrows). Umbilical cord. Fetus 6

Figure 29. Detail of Figure 28. Polymorphonuclear neutrophils and large foamy macrophages (arrows). Umbilical cord. Fetus 6


A
A
P
M



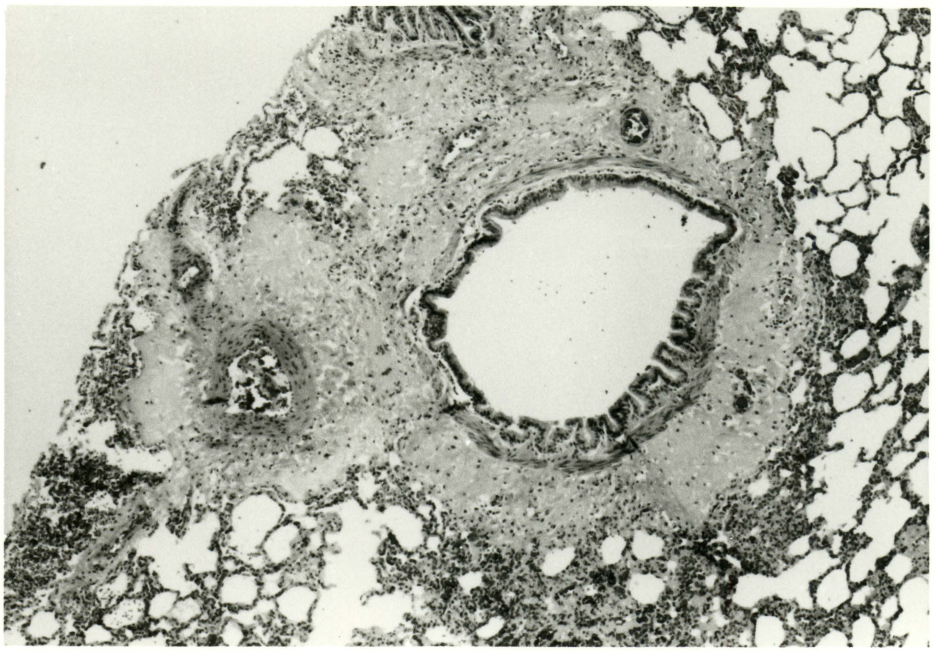
Horse No.	1	2	3	4	5	6	7	8
Lung	-	(+)	++	+	++	+	+	-
Uterus	-	-	-	-	-	-	++	-
Ovary	-	(+)	(+)	++	++	-	-	-
Liver	NE	-	NE	NE	+	-	-	-
Spleen	NE	(+)	+	+	-	+	-	-
L. node	-	+	NE	NE	++	+	NE	-

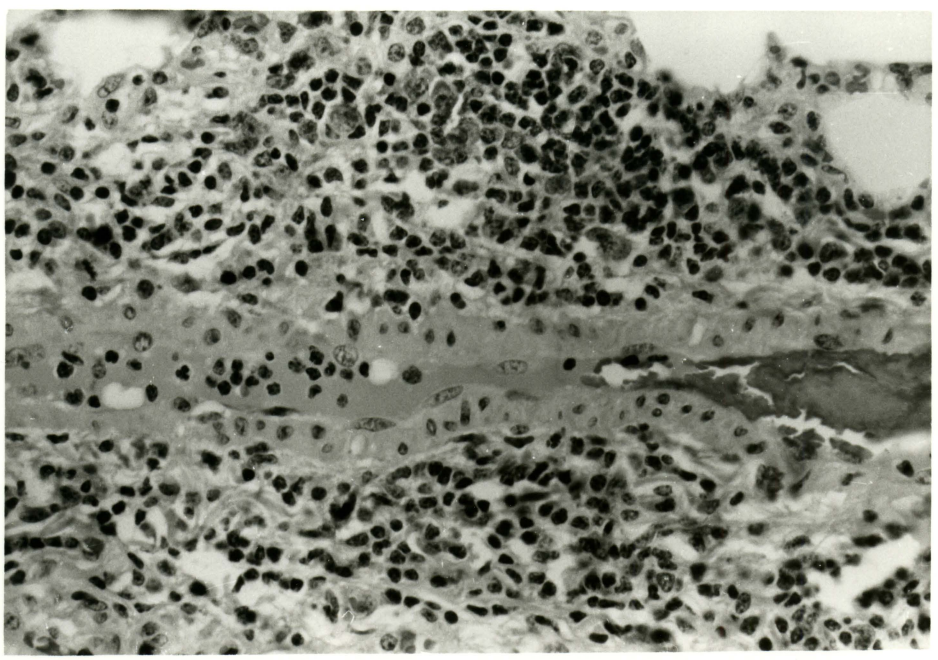
- = no lesion
(+) = some neutrophils scattered around blood vessels
+ = infiltration by inflammatory cells, localized edema
++ = blood vessel necrosis, extensive edema, tissue necrosis
NE = not examined

Figure 30. Distribution of lesions in mares

Figure 31. Lung. Homogeneous eosinophilic edema around a bronchiole and associated blood vessel. Mare 6

Figure 32. Monocytic and lymphocytic infiltration around a small artery. Lung, mare 7



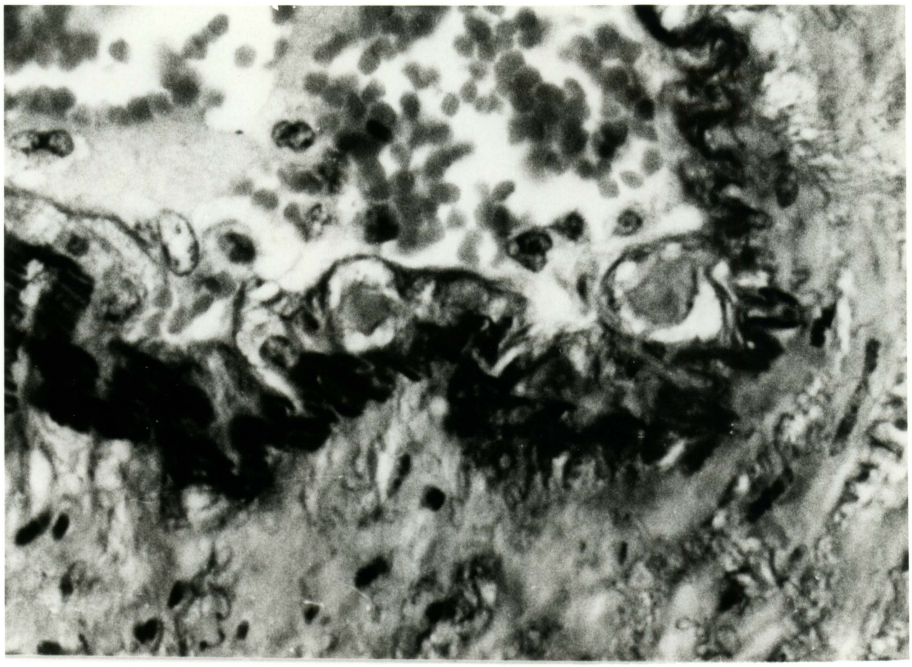


muscular arteries. Blood vessels of the lungs were variously affected in the same animal; some appeared intact, others had extensive necrosis. Edema was sometimes mixed with red blood cells or scattered lymphocytes and histocytes. Neutrophils were usually present in the lumen of blood vessels or in the vessel wall, the perivascular infiltration being almost exclusively histio-lymphocytic (Figure 32). In various organs, in small arteries, endothelial cells were swollen with large irregular hyaline globules previously described (Figure 33) (18,33). More advanced lesions of EVA were also seen in veins and arteries. Endothelium was sometimes absent and a layer of neutrophils covered the connective tissue of the intima. Occasional thrombosed vessels were present Figure 34 & 35).

The internal elastic lamina was disrupted in many blood vessels with disorganization, vacuolation, and necrosis of the media in the areas of rupture (Figure 36 & 37). Cell necrosis was prominent in the media of small muscular arteries. The necrotic cells were vacuolated and had karyorhectic nuclei (Figure 38). In some extensively affected vessels, most cells were necrotic and evidence of serum and blood leakage were found. Liver lesions were only observed in one horse (#5). Blood vessels of the triads were surrounded by monocytes, lymphocytes, and slight edema. Diffuse edema, hemorrhages, and infiltration by neutrophils were present in the spleen and lymph nodes of mares 2, 3, 4, 5, and 6.

Figure 33. Large muscular artery. Swollen endothelial cells. Note the large, irregular intimal bodies.

Figure 34. Intestine. Lamina propria. Small vein. Destruction of the endothelium, adhesion and infiltration of the wall by polymorphonuclear neutrophils



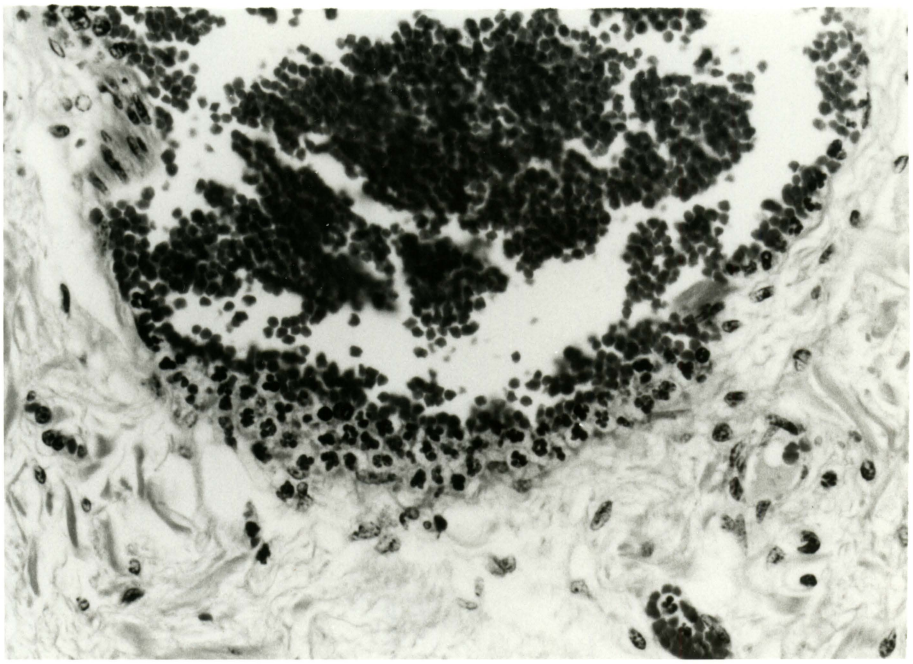
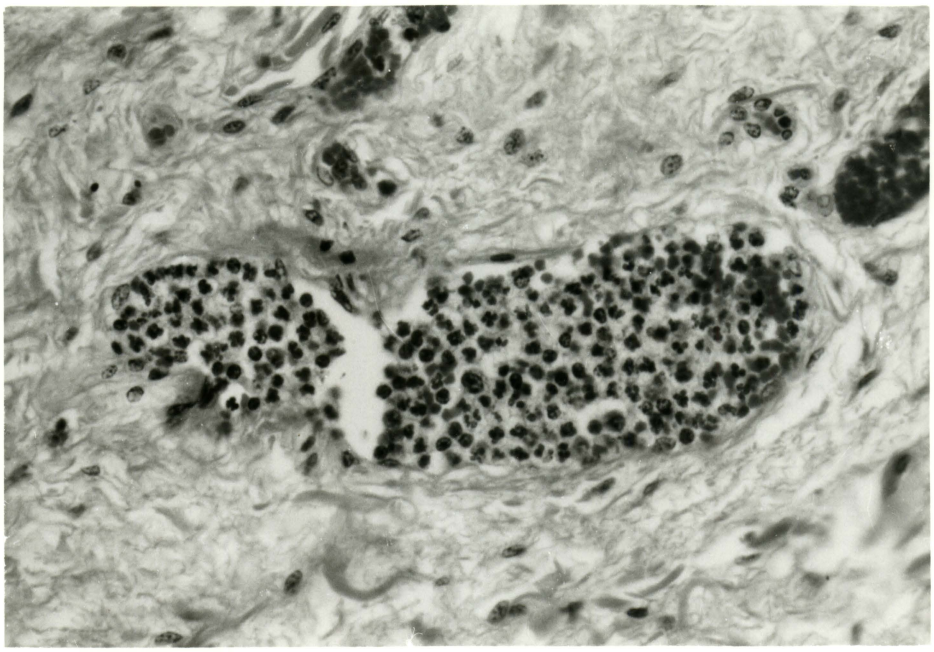


Figure 35. Intestine. Thrombosed vessel in the lamina propria

Figure 36. Lymph node. Small artery with focal rupture of the internal elastic lamina (elastin stain)



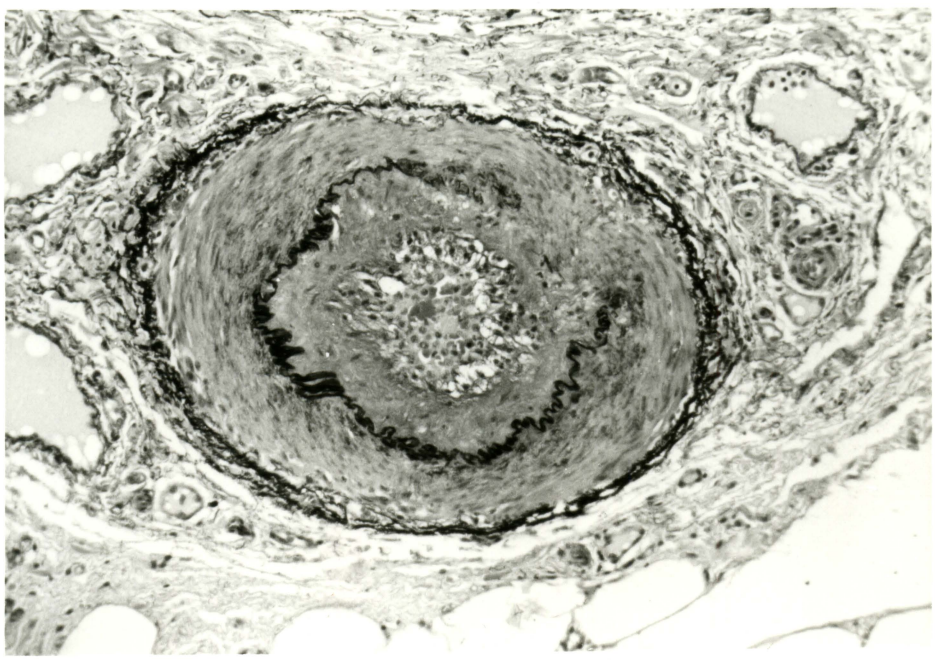
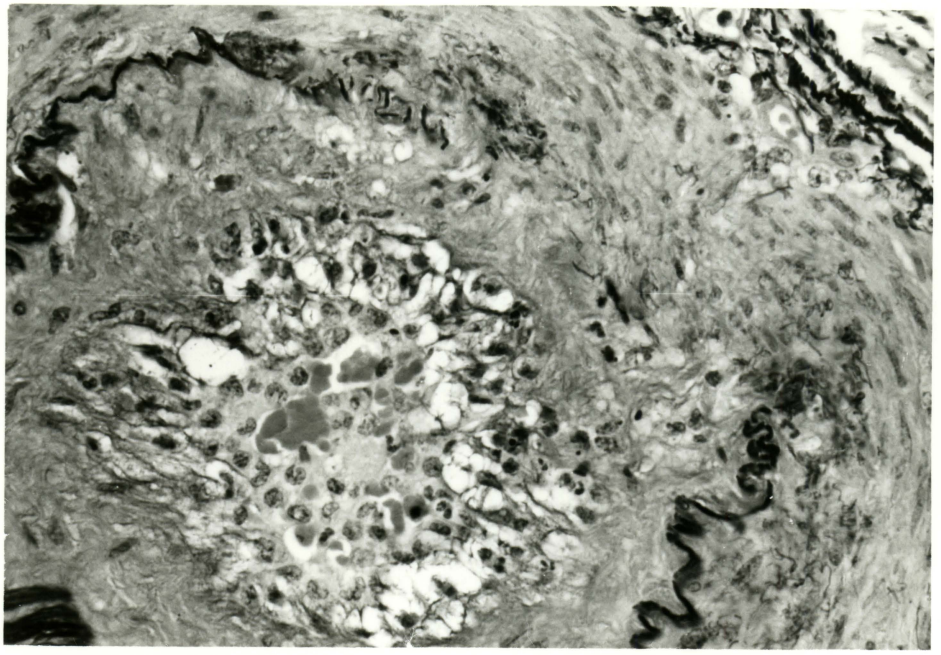
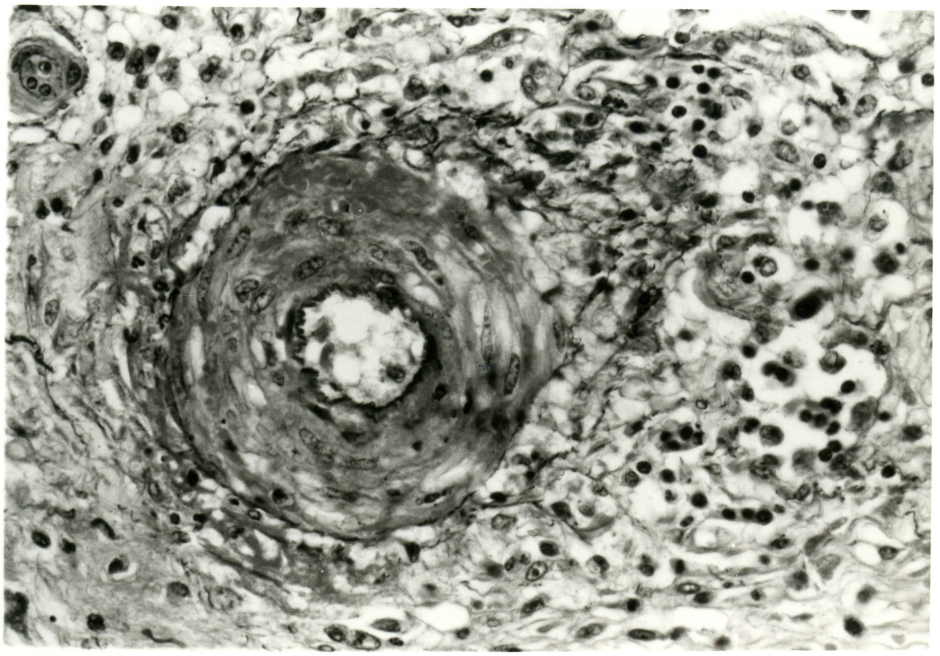


Figure 37. Magnification of Figure 19 showing endothelial swelling, internal elastic lamina rupture, media cells vacuolation and necrosis (elastin stain)

Figure 38. Small muscular artery. Media necrosis. Perivascular infiltration by round cells (elastin stain)





Cell cultures

On EMS cells, characteristic CPE and fluorescence were observed after infection with fetal material. The characteristics of this cell system's response to EVA virus have been published elsewhere (Carter, Coignoul, Cheville: submitted for publication). The virus was isolated from all samples of fetuses 6 and 7 and from the placenta of fetus 5. No attempt was made to isolate the virus from the placenta of the aborted fetus (Figure 39).

On EEK cells, the typical CPE consisted of focal cell necrosis. Distinct plaques were observed 24 hrs after passage of the virus. The CPE consisted of cell rounding and detachment. Cells had a refringent appearance by phase microscopy. By immunofluorescence, infected cells fluoresced brightly as soon as 24 hrs after infection. In cultures stained with Gram's crystal violet, the cytoplasm of the necrotic cells formed a deeply stained purple clumping around a karyorhectic or karyolytic nucleus. Multiple vacuoles were present in the perikaryon and long slender filamentous processes from the irregular border of the cytoplasm formed bridges with the adjacent cells. In the center of the plaque, the whole culture was replaced by thin filamentous strands of cytoplasm where small dark clumps marked the location of the nuclei. This cell system was used for virus multiplication, for virus titration, in an attempt to attenuate EVA virus for horse immunization, and for serum neutralization tests, because of its high growth rate and the early appearance of CPE.

Animal No.	1	2	3	4	5	6	7	8
Spleen	-	NP	-	N	-	+	+	+
Lung	-	NP	-	O T	-	+	+	+
Placenta	-	NP	-	P	+	+	NP	+
Testes/ovary	-	NP	-	R E G	-	+	+	+
Serum	NP	-	NP	N A N T	NP	+	NP	NP

+ = presence of virus
- = not isolated
NP = not performed

Figure 39. Virus isolation from fetal organs

Virus attenuation

A mare was infected intranasally with 200,000 FFU of a 10th passage of virulent virus on EEK. Two weeks later, an identical inoculum was given under the same conditions and, 3 weeks later, the animal was challenged with the virulent strain. This horse had no detectable serum neutralizing antibodies against EVA virus prior to infection. One week after the first challenge, the serum neutralization titer was 1/16. The titer rose to 1/256 after 2 weeks. Clinical signs and temperature were recorded daily for a week after each inoculation, WBC counts and PCV were recorded every day. No sign of EVA infection was present at any step of the immunization process.

DISCUSSION

In pregnant mares, EVA virus induced myometritis appears to be responsible for abortion. In the myometrium, necrosis and inflammatory foci were strictly limited to the bundles of smooth muscle. These lesions were identical to the fibrinoid necrosis induced in the media of small arteries by EVA virus. The idea of multifocal necrosis secondary to thromboembolisation is not relevant as confirmed by the absence of thrombi on samples stained for fibrin. Aggregates of ribosomes observed near the cell membrane in myometrial cells are similar to clusters of ribosomes present in cell culture of smooth muscle cells infected with the same virus. In normal smooth muscle cells, ribosomes are located in the vicinity of the nucleus.

Acute necrotizing miliary myometritis appears as a distinct lesion, without the systemic blood vessel involvement characteristic of EVA. Mare 7 was killed 8 days after experimental infection and no typical lesion of EVA was noted in blood vessels. It is unlikely that healing could have occurred in 8 days. On biopsy material from animals presenting the classical form of the disease, fibrinoid necrosis of the small arteries was maximum 10 days after infection with complete healing in 50 days (21).

Neutrophils in the endometrium and epithelial and glandular vacuolation of the placenta are not specific for EVA. They have been reported in mares delivering stillborn foals and after manual removal of retained placentae. These lesions have been related to failures in fetal blood

supply (43). In this study, endometrial lesions were found in all infected mares except mare 1. The lesions observed in mare 7 were more intense but not significantly different from the others. In all the mares, changes were located at the maternal side of placenta. The trophoblast layer appeared normal according to descriptions in the literature (38,39). We consider these lesions as not specific of EVA. They can be found in association with various conditions including abortion, distocia, and stillbirth (43).

Plasma progesterone drop before abortion can result from endometrial cell lesions but its importance in the mechanism of abortion is difficult to determine. Plasma progesterone levels vary extensively in pregnant mares according to the stage of gestation. Corpora lutea regress at 150 to 180 days pregnancy. Placenta is the source of progesterone during the second half of gestation (9,41) and endometrial lesions were quite extensive in mare 7. Determination of plasma progesterone is impaired by the presence in the blood of compounds interfering with accuracy (22). Small amounts of progesterone observed in the blood during the second part of gestation contrast with the high concentration in the umbilical cord (41).

Abortion is not due to a massive release of $PGF_2\alpha$ in the blood. During pregnancy, $PGF_2\alpha$ action is antagonistic to the action of progesterone. $PGF_2\alpha$ induces luteolysis in estrus cycle in human and large animal species (1,26,42). The major site of $PGF_2\alpha$ production during both estrus cycle and pregnancy is endometrium. Prostaglandins are also

released in tissue injury (26). $PGF_2\alpha$ has a very short life in serum. Its major metabolite, 13,14-dihydro-15-ketoprostaglandin $F_2\alpha$ is more stable and easier to determine (3).

A suggested mechanism for abortion is: [1] EVA virus multiplies in the myometrium resulting in necrotizing myometritis, [2] the lesion interferes with blood supply of endometrium and placenta, [3] progesterone production by placenta drops and abortion occurs. Altered endometrium not only induces progesterone decrease but it also fails to provide the indispensable nutrients to the fetus. Placenta detachment can be the major consequence of endometrial lesions, progesterone drop in the blood would then be a secondary event. A different or complementary mechanism consists in a massive release of prostaglandins. Prostaglandins are released by injured tissues (26). In the rat, an intrauterine injection of $PGF_2\alpha$ occurring in the same time as a drop in progesterone induces parturition (11). A local release of $PGF_2\alpha$ in the uterus or the involvement of another prostaglandin in the mechanism of abortion should also be considered.

Infected horses died within 36 hrs when their PCV reached values over the pre-inoculation level. In fatally infected horses, the initial decrease of PCV was followed by an abrupt rise; in the animals which recovered, PCV remained low during the whole period. The initial decrease matches with fever onset and can be due to serum hyperhydration. The sharp increase is attributed to an increased blood vessel permeability and subsequent serum leakage in the interstitium. The importance of PCV

in prognosis has not been mentioned in previous studies on blood variations after experimental EVA virus infection (7).

Clinical and pathological findings do not support disseminated intravascular coagulation as responsible for death in animals infected with EVA virus. Thrombocytes fell under physiologic levels in the terminal stage of the disease in only two horses. No evidence of fibrin clots was found at histology. A definitive conclusion on this point would require a kinetic study of blood factors I, II, V, VIII, XIII, RBC morphology, and platelet counts. Endotheliotropic viruses such as hog cholera, canine hepatitis, and human arboviruses can induce severe thrombocytopenia (48). The first site of virus replication in the cardiovascular system is the endothelial cell (21) and it is likely that platelet count drop is due to increased adhesion to the endothelial wall.

Hypovolemic shock due to extensive blood plasma losses seems to be the best explanation for death in equine viral arteritis. Blood glucose was over normal limits before inoculation in all the animals. Stress associated with manipulations for blood sampling can account for that observation. An intense hyperglycemia, reaching levels of 300 mg%, appeared right after infection. This can indicate a non-specific stress reaction due to a release of epinephrine and glucocorticosteroids, or, most likely, be a manifestation of shock.

Redistribution of PMN's from the circulating to the marginated pool may account for granulocytopenia. Studies on adherence of human poly-

morphonuclear neutrophils on bovine and human endothelial cells demonstrated an increased adhesiveness of WBC after infection of cells by polio or herpesvirus (32). Minimal white blood cell counts occurred 2 to 3 days postinoculation in the fatally infected horses, 5 to 6 days postinoculation in the 2 horses which survived. This difference can indicate the rapidity of virus multiplication and resulting endothelial damage in the 2 groups. Adhesion of neutrophils to the intima of blood vessels was observed in this study (Figure 34). Literature reports indicate that the leukopenia observed in EVA is both a neutropenia and a lymphopenia. According to the large number of lymphocytes present around the blood vessels (Figures 34 and 38), it is likely that lymphopenia also results from margination of lymphocytes from the vascular bed.

The lesions observed in fetuses were not related to EVA. Similar findings are described in normal foals at term. They are attributed to a break in the amniotic epithelium with penetration of epidermal debris, red blood cells, and meconium in the umbilical cord (46).

EVA virus does not produce any lesion that can be detected by histology and does not induce any defense mechanism in immunocompetent fetuses. The virus was recovered from all tissues collected from fetuses 6 and 7. These animals were at the 10th month of gestation and immunologically competent as shown by the granulomatous reaction elicited by allantoic fluid in the umbilical cord. This lack of reaction is

strikingly different from the reaction of the newborn animals. Foals are exceedingly sensitive to the disease.

Large hyaline globules seen in the endothelium of small arteries were intimal bodies. Intimal bodies are complex structures composed of a central mineralized core surrounded by intercellular material located in the subendothelial space of blood vessels. They appear to arise from subendothelial cells, are commonly found in horses, and have been associated with early degenerative events (33).

Passage of EVA virus on cell culture resulted in a complete loss of its pathogenicity. This is in contradiction with the literature. Reports indicate that a 25th passage of the virus in identical conditions is still able to induce clinical signs.

CONCLUSIONS

The major conclusion of this work is that EVA can exist under, at least, two different forms. A generalized systemic infection with panvasculitis, hypovolemia and death by hypovolemic shock is the only form consistently described. A second form of the disease is presented here as a metritis localized to the myometrium in pregnant animals. Reports on natural outbreaks of equine viral arteritis describe mild, transient clinical signs, rare fatalities, and abortion in 30 to 60% of pregnant mares (13,28). Experimental infections result in 30-60% mortalities with generalized lesions of blood vessel necrosis (7,28). These 2 types of disease parallel the descriptions of the present study. There is no evidence of more than one serotype of virus in this disease. Therefore, studies on virus resistance outside the organism, transmission from an animal to another and between herds should be undertaken.

Abortion in equine viral arteritis is associated with uterine lesions and maternal placenta modifications. Endometrium anoxia and destruction, subsequent loss of feto-maternal exchanges with a decrease in progesterone may represent the basic mechanism of abortion.

In animals clinically affected with EVA, PCV and total WBC counts provide a reliable way to anticipate the evolution. Striking rises in PCV and rapid fall in total WBC are associated with a poor prognosis.

SUMMARY

Seven pregnant pony mares were infected with equine arteritis virus. Six mares had clinical signs of EVA, five were found dead or were killed in agony. Systemic lesions of necrotizing vasculitis were observed in these horses, death was attributed to hypovolemic shock. During clinical survey, it appeared that PCV and total WBC counts provided good prognostic information.

The last mare recovered from clinical disease and aborted 9 days postinoculation. Lesions were restricted to the uterus. A multifocal necrotizing myometritis and placenta lesions were present. It was concluded that EVA can appear under two clinical forms, only one of these having been previously reported.

A mechanism for abortion was proposed, the destruction of endometrium resulting in a sharp decrease in serum progesterone and loss of feto-maternal exchanges. The virus was recovered from placenta and fetal membranes but no evidence of viral lesions were found. Ten passages of EVA virus on embryonic equine kidney primary cell culture resulted in a complete loss of pathogenicity. This finding is in contradiction to previous reports.

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