A Pathogenetic Study of the Early Connective Tissue Lesions of Viral Caprine Arthritis–Encephalitis

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Experiments were designed to correlate morphologic lesions with the presence of caprine arthritis-encephalitis virus (CAEV). Twenty-one cesarean-derived goat kids were infected with 10^6 to 10^7 $TCID_{50}$ of virus, killed sequentially, and examined for viral antigens by immunofluorescence, viral infectivity by isolation and titration, and morphologic changes by light microscopy. Fluorescent viral antigens were detected from 1 to 10 days postinoculation (DPI) and only in synovial cells. Virus was reisolated from several joints and from brain 0.5 to 79 DPI. Increases in synovial fluid cell counts were noted by 1 DPI, and morphologic changes in synovial membranes were present from 3 to 45 DPI. Joint lesions progressed from mild synovial cell hyperplasia and perivascular mononuclear cell infiltration to severe synovial cell hyperplasia and mononuclear cell infiltration with villous hypertrophy. Lesions elsewhere were mild, consisting only of perivascular mononuclear cell infiltrates. Eleven cesarean-derived control goats were negative for viral antigens, virus, and morphologic lesions. (Am J Pathol 1980, 99:257-278)

CAPRINE ARTHRITIS-ENCEPHALITIS (CAE) is a naturally occurring disease syndrome of domestic goats involving the central nervous system (CNS) and connective tissues, especially those associated with synovial-lined cavities. The chronic degenerative arthritis of CAE is most often seen in goats 1 year of age or older. The onset is usually insidious, although it can be abrupt, with acute articular swelling and pain. Salient clinical features of the disease include generalized wasting, depressed demeanor, and a highly variable course. The basic lesion is proliferative synovitis of joints, tendon sheaths, and bursae, characterized by villous hypertrophy, synovial cell hyperplasia, and infiltration by lymphocytes, macrophages, and plasma cells. Progression is accompanied by degenerative changes such as fibrosis, necrosis, and mineralization of synovial membranes as well as periarticular collagenous structures. Osteoporosis may lead to collapse of osseous subarticular structures and deformation of joints.[1] The CNS manifestation usually affects young goats and is accompanied by lesions morphologically similar to visna of sheep and postinfectious encephalitis of man.[2,3] A retrovirus (CAEV) isolated from joints

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and brains of goats with CAE is the cause of both the arthritis and the encephalomyelitis.[1]

Several retrovirus infections of animals are associated with chronic inflammatory and degenerative diseases. New Zealand black mouse disease, a model for systemic lupus erythematosus,[4] visna, a chronic progressive neurologic disorder of sheep,[5] lower motor neuron disease of lymphoma prone mice,[6] and equine infectious anemia, a chronic recurrent hemolytic disease of horses,[7] exemplify this group of persistent virus infections. As a new member of this group, CAE affords an opportunity for investigation of a unique host–retrovirus relationship resulting in chronic connective tissue disease. The purpose of this study was to begin defining this relationship by examining the character and kinetics of the early connective tissue lesions relative to the presence of the virus.

Materials and Methods

Experimental Design

The purpose of Experiment I was to determine the pathologic changes and distribution of viral antigens during the early phases of infection by CAEV. However, no viral antigens could be demonstrated in any of the tissues in Experiment I. Because of the seasonal availability of neonatal goats, Experiment II was not conducted until a year later, in which time technical improvements made in immunofluorescence (IF) verified the negative IF results of Experiment I. In Experiment II, a more eleborate set of samples was collected for IF, particularly of synovial membranes and synovial fluid cells, and titrations of virus in synovial fluid were added. The basic experimental designs being similar, the results were considered together.

Experiment I

In Experiment I, 19 cesarean-derived kid goats were distributed into 3 groups according to sex, breed, and familial relationships. Group 1 consisted of 10 animals 7–9 days of age inoculated with 1 ml of 75-63 virus-infected medium intravenously and 0.25 ml in the left radiocarpal joint. They were killed in pairs at 6, 13, 19 (18 and 20), 34, and 45 days postinoculation (DPI). Three kids in Group 2 were similarly inoculated with control cell culture medium and killed at 27, 29, and 39 DPI. Six uninoculated animals in Group 3 were killed at 23, 29, 39, 45, 52, and 68 days of age. Immediately after euthanasia by anesthetic overdose, a blood sample was taken, and tissues were collected for histopathologic studies, IF, and virus isolation. The following tissues were fixed in 10% phosphate buffered formalin for histopathologic studies: brain, spinal cord, lung, liver, thymus, spleen, kidney, adrenal gland, duodenum, jejunum, ileum, colon, skeletal muscle, cardiac muscle, parotid salivary gland, mammary gland, synovial membranes from both carpi, and hilar, hepatic, mesenteric, and axillary lymph nodes. Synovial membranes and articular cartilage from both carpi, axillary lymph nodes, mammary gland, kidney, thymus, spleen, lung, brain, spinal cord, choroid plexus, and bone marrow (bone marrow from 45 DPI goats only) were frozen in liquid nitrogen for IF.

Experiment II

Thirteen cesarean-derived goat kids from 2 to 14 days of age were used in this experiment. Ten were inoculated with virus-infected cell medium, 0.25 ml in the left radiocarpal

joint and 1.75 ml intravenously. Three of these animals were killed at 3 DPI, 2 at 6, 3 at 9, and 2 at 13 DPI. Two control kids were similarly inoculated with control cell culture medium and killed at 6 and 13 DPI. The tissues collected and processed for histologic examination were the same as in Experiment I, with the addition of prescapular lymph nodes, tibiotarsal joints, thyroid, pancreas, gonads, abomasum, and bone marrow. For IF, both radiocarpal synovial membranes (6 sections each), prescapular lymph nodes, jejunum, ileum, abomasum, bone marrow, myocardium, mesenteric lymph node, thoracic and sacral spinal cord, and synovial fluid cells from both left and right radiocarpal joints were collected in addition to those listed for Experiment I.

In addition to the main experiment, another single kid was infected by injection of the same amount of virus-infected medium intra-articularly in both radiocarpal joints, and sampled sequentially. The purpose was to monitor the presence of IF viral antigens, to study the sequential morphologic changes of synovial fluid cells, and to determine the persistence of virus infectivity. Synovial fluid was aspirated by arthrocentesis from the left radiocarpal joint at 0.5 DPI, from the right at 1 DPI, and from both at 2, 4, 6, 8, 10, 14, 18, 35, 49, and 79 DPI. Samples were processed in the same manner as described below for virus titration. Synovial fluid cells pelleted from the fluid were fixed onto glass slides with acetone for IF or glutaraldehyde for histologic examination as described below.

Animals

Because of the high prevalence of natural infection with CAEV, which can be prevented by cesarean section,[1] experimental kid goats were obtained by cesarean section and separated from their dams. Immunoglobulin prepared by 50% ammonium sulfate precipitation of serum from clinically normal goats was administered subcutaneously on the first or second day of life in lieu of colostrum. In Experiment I, the immunoglobulin solution was treated with ether and heated to 56 C for 30 minutes to inactivate any infectious CAEV. In Experiment II, the immunoglobulin preparation was derived from the serum of cesarean-derived goats 6–8 months of age that were free of CAEV antibody by immunodiffusion. Control kids were housed separately from inoculated kids, and all were bottle-fed pasteurized cow milk and provided hay, grain, and water *ad libitum*.

Virus Inoculum

The prototype strain (75-63) of CAEV used for inoculation was originally isolated from an adult goat with spontaneous arthritis by explantation of tissue from affected joints as described elsewhere.[1] The inoculum for Experiment I consisted of unfrozen medium from synovial cell cultures infected with the 11th passage of the virus. The infectivity titer was $10^{6.2}$ $TCID_{50}$/ml after 1 freeze–thaw. The inoculum for Experiment II was from a virus pool of the same passage history frozen at −70 C; it had an infectivity titer of $10^{6.5}$ $TCID_{50}$/ml. The control inoculum was medium from uninfected cultures.

Virus Isolation and Titration

In Experiment I, virus was isolated from tissue samples of the experimental goats by explantation. Aseptically collected samples were minced into 1- to 2-mm fragments, washed and planted in plastic flasks in a minimal volume of Dulbecco's modified minimal essential medium (DMEM) with 20% fetal calf serum and 100 μg/ml Fungizone (Squibb & Sons, Princeton, NJ). Following cellular outgrowth from the fragments, virus replication was detected by the appearance of the characteristic cytopathic effect.[1] Cultures were passaged three times before being considered negative.

Before virus titration, synovial fluid samples (Experiment II) were clarified in a Beckman microfuge at 9400*g* for 2 minutes. The supernatant was removed for titration, and the cell pellet was reserved for IF. Serial doubling dilutions were made in DMEM plus 1%

BSA and antibiotics. Fetal goat synovial membrane cells in 96-well microtiter plates (Linbro, Hamden, CN) were pretreated with 4 μg/ml polybrene for 30 minutes. Five wells were inoculated with 0.1 ml of each two-fold dilution. After a 2-hour adsorption period, 0.1 ml of growth medium was added to each well. Cultures were maintained at 37 C in a humidified, 5–10% CO_2 atmosphere and monitored for the appearance of CPE. Viral titers were determined from 50% end points ($TCID_{50}$) by the method of Reed and Muench.[8]

Immunofluorescence

For fluorescent staining, synovial membrane cells were grown on coverslips or in compartmented slides (Lab-Tek, Miles Laboratories) and fixed in acetone at 22 C for 15 minutes or at −20 C for 2 hours. These preparations were used immediately or stored at −70 C. Goat tissues frozen in liquid nitrogen were cut 4 to 6 μ thick on a cryostat and fixed in the same way. Cells pelleted from synovial fluid at 9400g for 2 minutes were washed twice in DMEM with 10% fetal calf serum, divided into 5 aliquots, and centrifuged (Cytospin, Shandon-Southern Products, Ltd., Cheshire, England) onto separate slides. One slide was fixed in glutaraldehyde for morphologic examination (see histologic techniques); the rest were fixed in acetone as described above.

Fluorescein-labeled immunoglobulin conjugates were prepared from 2 different sources of serum: 1) a naturally infected but clinically normal goat (76-62) immunized with purified CAEV antigens, 2) a pool of serum from 4 cesarean-derived goats, containing no detectable precipitating antibody to ether-extracted CAEV. Gamma globulins were derived by serial 50% and 40% ammonium sulfate precipitations, dialyzed, labeled with fluorescein isothiocyanate, and fractionated from DEAE cellulose by stepwise elution with 0.05, 0.1, 0.2, and 0.4 M NaCl as previously described.[9] Each fraction was tested for brilliance and specificity of staining. The anti-CAEV conjugate had an F : P ratio of 0.97 and a protein concentration of 3.2 mg/ml, as determined by the method of Lowry.[10] The negative control conjugate had an F : P ratio of 1.4 and a protein concentration of 3.7 mg/ml. Both conjugates were used for staining at a protein concentration of approximately 0.75 mg/ml.

Controls for specificity included the following. The anti-CAEV conjugate was applied to CAEV-infected and control tissue culture cells and goat tissues. Experiments to inhibit staining of the anti-CAEV conjugate by absorption with purified CAEV and with equine infectious anemia virus were devised. Both viruses were purified and concentrated as described [11] and degraded by ether extraction. Seventy-six micrograms of CAEV protein in 40 μl of diluent was added to 1 ml of the working concentration of anti-CAEV conjugate. One-hundred and forty μg of equine infectious anemia viral protein in 40 μl of diluent was added to a second 1-ml sample of anti-CAEV conjugate. The two mixtures were incubated for 1 hour at 22 C and then kept overnight at 4 C. They were then centrifuged at 1000g for 20 minutes before staining. Competitive inhibition was attempted by pretreatment of substrates for 30 minutes at 37 C with serums with or without anti-CAEV antibody activity.

All staining was done in duplicate at 37 C for 30 minutes, 1 of the duplicates with anti-CAEV conjugate and the other with control conjugate. They were then washed three times in PBS for 2 minutes each time and mounted in phosphate-buffered saline (PBS) containing 10% glycerol. The samples were examined on a Zeiss fluorescence microscope using incident illumination from a mercury vapor lamp.

Histologic Technique

Formalin-fixed tissues from both experiments were embedded in paraffin, sectioned at 4–6 μ, and stained with hematoxylin and eosin (H&E) for histologic examination. Synovial fluid cells and cryostat tissue sections adjacent to sections with positive fluorescence were fixed with glutaraldehyde and stained with H&E. Selected tissue sections or synovial fluid cells with positive fluorescence were restained with H&E. Phase microscopy was employed when necessary to facilitate identification of the cells.

Antibody Assay

Anti-CAEV antibody was assayed by an agar gel immunodiffusion method [12] using purified CAEV that had been ether-extracted.

Results

The results of Experiments I and II are presented simultaneously except where significant differences in their protocols or results warrant separate description.

Morphologic Changes

Distinctive changes were found in goats inoculated with the virus. The most consistent finding was infiltration of the synovial membranes and perivasculature of other tissues by mononuclear inflammatory cells. These lesions were most severe in synovial-lined structures, particularly inoculated joints, and were progressive. None of these changes were found in control animals (Tables 1 and 2).

At 3 DPI the lesions were very mild and visible only in inoculated joints. Edema separated the synovial membrane from its underlying connective tissue. Inflammatory cells accumulated focally in the synovial membrane and were often oriented around vessels, especially in villi. Approximately 30% of the inflammatory cells were polymorphonuclear (PMN), and the remainder were macrophage-like cells. Synovial membrane cells were enlarged and rounded, and had eosinophilic cytoplasm.

By 6 DPI numbers of mononuclear inflammatory cells had increased in the inoculated joints of 2 of 4 kids. Polymorphonuclear cell numbers had declined, and perivascular mononuclear infiltrates were appreciably more widespread and heavier than at 3 DPI (Figure 2). The infiltrates of 1 kid contained significant numbers of eosinophils. Two of the 4 goats killed at 6 days had no discernible inflammatory cell infiltrates, only hypertrophy and eosinophilia of synovial membrane cells. This observation, however, may have been a sampling artifact due to the distinctly focal nature of the inflammatory cell infiltrates at this stage.

By 9 and 13 DPI, the lesions had increased in severity and prevalence in inoculated joints. Joints of all 7 animals contained mononuclear inflammatory cell infiltrates, and most had some degree of synovial membrane cell hyperplasia. Mild infiltrates were present in the contralateral, uninoculated joints of 3 of 7 infected kids and were composed mainly of macrophages and PMNs.

At 18 and 20 DPI, joint capsules were grossly distended with excess synovial fluid. Microscopically, villi were thickened by accumulations of macrophages, lymphocytes, and plasma cells. At the surface, many plump

Table 1—Experiment I: Presence of Histologic Lesions, Viral Antigens, Virus, and Antibody in Control Kids and Kids Inoculated with Caprine Arthritis–Encephalitis Virus

Goat number	DPI	Histologic lesions*			Viral antigens† (IF)	Infectious virus‡	Antibody to CAEV
		Joints		Other organs			
		Inoculated	Uninoculated				
75-63 CAEV§							
1	6	+	–	–	–	ND	–
2	6	–	–	–	–	ND	–
3	13	++	–	–	–	IJ	–
4	13	++	–	–	–	IJ, BR	–
5	18	+++	+	+	–	UJ	–
6	20	+++	–	–	–	IJ	–
7	34	+++	–	–	–	IJ, UJ	+
8	34	++++	+++	+	–	UJ	+
9	45	++	+	+	–	UJ	+
10	45	++++	+	+	–	IJ	+
Control medium§							
11–	27–						
13	39	–	–	–	–	–	–
Uninoculated							
14	23 ‖	–	–	–	ND	–	–
15–	29–						
19	68	–	–	–	ND	ND	–

* –, no lesions; +, focal perivascular mononuclear infiltrates; ++, more general mononuclear cell infiltration and proliferation of synovial cells; +++, villous hypertrophy and intense mononuclear accumulations; ++++, the foregoing, with the addition of lymphoid follicles, hemorrhage, and fibrin within the synovial membrane. Inoculated and uninoculated columns indicate lesions in joints.

† Only tissue sections, not synovial fluid cells, were stained.

‡ ND = not done, IJ = inoculated joint, B = brain, UJ = uninoculated joint. Isolation was by explantation.

§ Inoculation was by both intra-articular and intravenous routes.

‖ Age in days at sacrifice.

Table 2—Experiment II: Presence of Histologic Lesions, Viral Antigens, and Virus in the Joints of Control Kids and Kids Inoculated with Caprine Arthritis–Encephalitis Virus

		Histologic lesions in joints*		Viral antigens in joints†		Infectious virus in joint fluid‡	
Goat number	DPI	Inoculated joint	Uninoculated joint	Inoculated joint	Uninoculated joint	Inoculated joint	Uninoculated joint
75-63 CAEV§ inoculated							
20	3	+	–	+	–	$10^{3.0}$	$<10^{1}$
21	3	+	–	+	–	$10^{3.6}$	$10^{2.4}$
22	3	+	–	+	–	ND ‖	ND
23	6	+	+	+	–	$10^{5.0}$	$10^{1.9}$
24	6	+	–	+	–	$10^{4.5}$	$10^{1.9}$
25	9	+	+	–	–	$<10^{1}$	$<10^{1}$
26	9	++	+	+	–	ND	ND
27	9	++	–	+	–	$10^{2.7}$	$10^{2.3}$
28	13	++	+	–	–	$10^{3.6}$	$<10^{1}$
29	13	++	+	–	–	$10^{3.5}$	$10^{2.2}$
Control medium§ inoculated							
30	6	–	–	–	–	$<10^{1}$	$<10^{1}$
31	13	–	–	–	–	$<10^{1}$	$<10^{1}$

* –, no lesions; +, focal subsynovial and perivascular mononuclear and accumulations, eosinophilia of synovial membrane cells; ++, more general mononuclear cell infiltration and mild proliferation of synovial membrane cells. Lesions were not found in other organs.

† IF was attempted on numerous tissues; + indicates positive fluorescence for CAEV antigens in synovial fluid cells or synovial membrane cells or both.

‡ $TCID_{50}$/ml in synovial fluid.

§ Inoculation was via both intra-articular and intravenous routes.

‖ ND = not done.

eosinophilic synovial membrane cells, sometimes with pyknotic nuclei, were scarcely attached to the underlying stroma (Figure 3). Vessels were surrounded by heavy cuffs of inflammatory cells, and hypertrophied endothelial cells appeared to encroach on vascular lumena. These changes were present throughout most of the inoculated joints and even extended to synovial linings of tendons and sheaths adjacent to the joint. One of the 2 kids had a small perivascular cuff in a tendon of an uninoculated joint and a small focus of mononuclear cells in the liver.

The severity of lesions, although variable, continued to progress between 20 and 34 DPI to the point that joint changes were grossly evident in 2 of the 4 goats killed at 34 and 45 DPI. The inoculated joints of the 2 most severely affected kids (1 each from 34 and 45 DPI) were swollen and contained excessive, turbid synovial fluid. The synovial membrane was tan in both kids, and petechiae were scattered over the surface of the membrane in 1 kid. Numerous petechial hemorrhages and large areas of fibrinous exudation were histologically evident on and beneath the synovial membrane (Figure 4). So numerous were the macrophages, lymphocytes, and plasma cells that the enlarged synovial villi fused and began to resemble lymphoid tissue complete with lymphoid follicles and germinal centers (Figure 5). There was marked thickening of villi and mononuclear cell infiltration in an uninoculated joint as well as occasional perivascular infiltrates of lymphocytes and plasma cells in the cervical spinal cord, kidney, and epicardium in one of the kids killed at 34 DPI. Definite though mild lesions were present in the uninoculated joints of both kids killed at 45 DPI. Perivascular mononuclear cell infiltrates were found in the subcutis over one carpus and in the brain, kidney, and epicardium (Table 1).

The morphologic changes in synovial fluid cells were examined in Experiment II only. The synovial fluid of inoculated joints at 2 DPI had low numbers of plump eosinophilic cells with dense nuclei morphologically identical to synovial membrane lining cells, often appearing in clumps of 5 to 50 cells. A few PMNs were also present. By 3 DPI, numerous PMNs (approximately 25% of the total) and a few large, vacuolar cells resembling macrophages were present in addition to the plump eosinophilic cells. By 6 DPI the cell population had changed: very few PMNs remained; yet large numbers of foamy cells and a few cells with nonvacuolar cytoplasm were present. Phagocytosis of other cells by the large vacuolar cells was occasionally seen. The population of synovial fluid cells in goats killed at 9 DPI was similar, but phagocytosis of cells was more common (Figure 6). By 13 DPI a decline in phagocytosis and foamy cells had begun. Nevertheless, large numbers of cells with dense eosinophilic

cytoplasm persisted in the synovial fluid of inoculated joints at least up to 18 DPI.

One infected goat killed at 9 DPI had significant numbers of cells in the uninoculated joint. The types and distribution of the cells were similar to what was seen in inoculated joints at 3–6 DPI.

Immunofluorescence

The anti-CAEV conjugate produced specific particulate intracytoplasmic and intranuclear fluorescence in CAEV-infected fetal synovial membrane cells in culture (Figure 7); uninfected cells did not stain. Absorption of the conjugate by purified, degraded CAEV removed the staining activity of the conjugate, but absorption by purified degraded equine infectious anemia virus did not. Treatment of the substrate cells with unlabeled anti-CAEV serum blocked approximately 75% of the intensity of staining, but negative serum had no effect. The negative control conjugate was consistently negative on infected cells.

Five-hundred tissue sections and 36 synovial fluid cell samples were examined using the anti-CAEV conjugate. Specific fluorescence was found only in virus-inoculated joints less than 11 DPI (Tables 1 and 2). In Goat 79-17, from which fluid was repeatedly sampled, fluorescence first appeared in a few synovial fluid cells at 1 DPI and persisted only through 10 DPI (Table 3). Identical staining was present in the synovial fluid cells of all kids killed at 3 and 6 DPI, and 1 kid killed at 9 DPI in Experiment I.

Table 3—Experiment II: Presence of Viral Antigens in Synovial Fluid Cells and Persistence of Infectivity in the Fluid

DPI*	Viral antigens in synovial fluid cells†	Infectious virus in joint fluid
0.5	–	$10^{4.6}$‡
1	+	$10^{3.6}$
2	+	ND
4	+	ND
6	+	ND
8	+	ND
10	+	ND
14	–	ND
18	–	ND
35	ND	$10^{3.0}$, $10^{3.6}$
49	–	$10^{2.8}$, $10^{2.9}$
79	–	$10^{2.5}$, $10^{2.9}$

* This goat was not killed; samples were drawn repeatedly from the joints at the DPI listed.

† Detected by IF.

‡ Both joints were inoculated with CAEV. The left radiocarpal synovial fluid was sampled at .5 and the right at 1.0 DPI, and from 35 to 79 DPI both joints were sampled. Refer to Table 2 for titers 3–13 DPI.

Typically, it was very faint and finely granular, 1–5% of the cells (Figure 8); however, the synovial fluid cells of 1 goat killed at 6 DPI exhibited particularly bright cytoplasmic and nuclear fluorescence in 50–70% of cells (Figure 9). The morphologic features of these cells were established by restaining the cells shown in Figure 9 with H&E and examining them with phase-contrast microscopy (Figure 10). The smaller round cells with eosinophilic cytoplasm showed mostly nuclear staining, whereas the large foamy cell showed both intranuclear and intracytoplasmic fluorescence. The fluorescence in synovial fluid cells met the same criteria of specificity as the fluorescence in cultured cells.

Viral antigen was demonstrated by IF in large cells with round nuclei attached to the surface of the synovial membrane in 4 goats, 2 from 6 DPI and 2 from 9 DPI. These cells usually appeared in foci on or very near the surface of the synovial membrane, particularly on villi. Often, only solitary cells would fluoresce (Figure 11), but occasionally several could be seen in one field (Figure 12). Because of their morphologic characteristics and location on the surface of the membrane, these cells were presumed to be synovial membrane lining cells. Nevertheless, it is conceivable that some other cell with a similar form, perhaps a macrophage, might be infected at the synovial membrane surfaces as well.

Virus Isolation and Titrations

Caprine arthritis–encephalitis virus was reisolated by explantation from all of the 8 infected goats in Experiment I from which isolation was attempted. Five isolates were derived from inoculated joints, 4 from uninoculated joints, and 1 from brain. Efforts to isolate virus from 4 of the control kids failed (Table 1).

In Experiment II, infectivity titers were determined from synovial fluids. Goat 79-17 was sequentially sampled at 0.5, 1, 35, 49, and 79 DPI. In goats that were killed, samples were collected from 2 goats each day at 3, 6, 9, and 13 DPI. The virus titers are recorded in Tables 2 and 3. Briefly, titers from $<10^1$ to $10^{5.0}$ were found with an apparent peak at 6 DPI. Virus was still detectable at low titer at 79 DPI in Goat 79-17. Low titers (between 10^1 and 10^3 $TCID_{50}$) were found in uninoculated joints, confirming that the infection had spread, apparently via the blood vascular system. These data indicate that the inoculated joints contained more infectious virus than the uninoculated joint. There was somewhat of a cyclic pattern to the infectivity with respect to time, but whether these fluctuations are specious or significant awaits further experimentation with more animals. No virus was obtained from the 2 control kids.

Antibody Assays

Antibody was detectable by immunodiffusion in all 4 goats killed 34 and 45 DPI, but not in goats killed before 34 DPI. No antibody was detected in any of the control kids.

Discussion

Early experiments showed that arthritis could be induced within 2–7 months after inoculation of clinically normal goats with synovial fluids or tissue homogenates from goats with natural CAE.[13] The early experiments were done on adult goats that, though clinically normal, may have been carriers of the virus. The present experiments using cesarean-derived goats demonstrate conclusively that CAEV (75-63) plays a primary etiologic role in the early inflammatory lesions. Nineteen of 20 virus-inoculated kids developed lesions. Caprine arthritis–encephalitis virus was consistently isolated from inoculated kids, viral antigens were present within the lesions very early in infection, and anti-CAEV antibody was demonstrated in 4 of the virus-infected kids. Soon after infection, before the onset of clinical signs, viral antigens are detectable for a short period but are undetectable by the time histologic lesions are well developed. Proliferative synovitis and mononuclear cell infiltration characterize the early connective tissue lesions of natural CAE, before the onset of degenerative changes associated with clinically apparent disease.

The cesarean-derived control goats were uniformly negative for lesions, infectious virus, viral antigens, and anti-CAEV antibody. These findings confirm not only the etiologic role of CAEV but also suggest that the natural mode of transmission may not be primarily vertical. Since these kids were cesarean-derived from CAEV-infected does, the infection is probably transmitted horizontally.[1]

The difference in severity of lesions between inoculated and uninoculated joints indicates a close relationship between the amount of virus present and lesion production (Tables 1 and 2). Within a given goat, the inoculated joint consistently contained more severe lesions, higher virus titers, and more immunofluorescent viral antigen. A correlation between severity of lesions and virus isolations has also been noted in visna of sheep.[14] Among individuals, however, this relationship had a tendency to break down. That is, some goats had worse lesions in the uninoculated joints than did others in inoculated joints, suggesting that other factors such as genetic differences between goats may also influence the severity of the lesions.

Since the present experiments were some of the first to use cell culture

virus and their purpose, in part, was to describe the early lesions in the disease, we learned very little else about the relationship between dose of virus and lesion development. The doses of $10^{6.2}$ and $10^{6.5}$ $TCID_{50}$ were chosen simply because that is what the cultures produced. Our choice of intra-articular and intravenous routes simultaneously was to increase the chances of producing lesions. Certainly future experiments should be designed to determine the effects of dose and route on infection and lesions. Early experiments, however, using brain homogenates as an inoculum utilized intracerebral and intraperitoneal routes, and both brain and joint lesions developed. It is strongly suspected as well that the natural route of horizontal transmission is oral. Therefore, it appears that several routes of infection are possible.

The data suggest that there may be an *in vivo* preference of CAEV for synovial membrane cells. Since the severity of lesions appears to be related to the amount of virus, it follows that the tissue consistently developing the most severe lesions may be the one in which the virus replicates best. In the present experiments, the uninoculated joint clearly developed more extensive lesions than any other tissue, even though initially it presumably received an equal dose of virus via the bloodstream. This finding suggests tropism for joints. In the limited number of samples in Experiment I, virus isolates were more consistently obtained from the uninoculated joint than from the brain, a finding further supporting a preference for the synovial tissue. This interpretation is supported by the demonstration of viral antigens in synovial membrane cells and synovial cells. However, since these fluorescent viral antigens were detected only in inoculated joints, it must be considered that their presence may have been a function of at least three alternate possibilities—1) an artifact of an intra-articular inoculation, 2) an exceptionally high dose of virus leading to greater replication, or 3) phagocytosis and concentration of viral antigens in macrophages—and not a reflection of tissue tropism per se. With respect to the third possibility, the rough coincidence of the peaks of viral infectivity in synovial fluid and viral fluorescence is an argument against it (Tables 2 and 3).

The apparent discrepancy in results of IF between Experiments I and II can most readily be explained by differences in the thoroughness of sampling. In Experiment I, only 1 or 2 blocks of synovial membrane were examined from each joint, whereas 6 were examined in Experiment II. The distinctly focal nature of fluorescent cell distribution in synovial membranes from Experiment II make it obvious in retrospect that 1 or 2 pieces were an inadequate sample. Furthermore, synovial fluid cells, which accounted for most of the fluorescence in Experiment II, were not examined

in Experiment I. Another potential factor was the presence of small amounts of anti-CAEV antibody from the gamma globulin preparations administered at birth to the goats used in Experiment I. This antibody could have played some role in reducing the expression of viral antigens to below the threshold of IF detection.

A close correlation between fluorescent viral antigens and infectious virus in synovial fluid was observed in inoculated joints between 3 and 9 DPI. In contrast, after 10 DPI, viral antigens could not be detected in synovial fluid or membrane cells, even though virus was still isolated from the fluid through 79 DPI. These findings may indicate that virion production in infected cells does not depend on IF-detectable levels of viral proteins. A precedent for this has been observed *in vitro* in equine fibroblasts infected with equine infectious anemia virus.[7] Another possibility is that virus replication in synovial fluid cells is minimal or nonexistent until the cells are placed in culture. The lack of fluorescent antigens in synovial cells of infected joints is apparently long-lived, because fluorescent viral antigens were also undetectable in the synovial fluid cells of 8 adult goats infected with CAEV for 9 months.[13] Lack of IF-detectable viral antigens in infected cells is also the rule in sheep infected with visna virus.[15–18]

The presence of virus in synovial fluid at 79 DPI indicates that host defense mechanisms are at least partially ineffective in controlling the infection. The intense mononuclear cell infiltrates and apparent proliferation of lymphoid elements (Figure 5) suggest that local antigenic stimulation takes place in the synovial membrane. The reason(s) host effector mechanisms do not control the infection in CAE is at present unclear. In visna and equine infectious anemia virus infection (both retroviruses) antigenic drift of viral surface glycoproteins results in viral escape from neutralization.[16,17,19,20] Whether this is the case in CAE awaits further experimentation.

The lymphoid component of the synovial membrane and sustained immune response to virion antigens also suggest that the synovial lesions may be immunologically induced by chronic antigenic stimulation. Expression of virus-coded or -induced antigens on the surfaces of infected cells[21–23] and/or virion production may be responsible. More sensitive methods of detecting antigens and measuring the specificity and magnitude of local immune response in the joint will be necessary to more accurately define the mechanisms of the synovitis.

There are similarities between clinical CAE[1] and the chronic arthritides of man, particularly rheumatoid arthritis. The early lesions of rheumatoid arthritis and CAE bear some resemblance to each other as well: perivascular distribution of lymphoid cells, proliferation of synovial lining

cells, fibrinous exudation, and lymphoid follicle formation in the synovial membrane are common to both diseases.[24-27] Exhaustive but largely fruitless attempts to isolate or demonstrate viruses in rheumatoid arthritis [28-35] suggest that the morphologic similarities may only reflect common pathologic responses to different inciting agents. In spite of this, experimental induction of synovitis with a retrovirus permits investigation of a new dimension in arthritis heretofore unexplored and may open new doors to the understanding of how a persistent virus infection might induce chronic arthritis in man.

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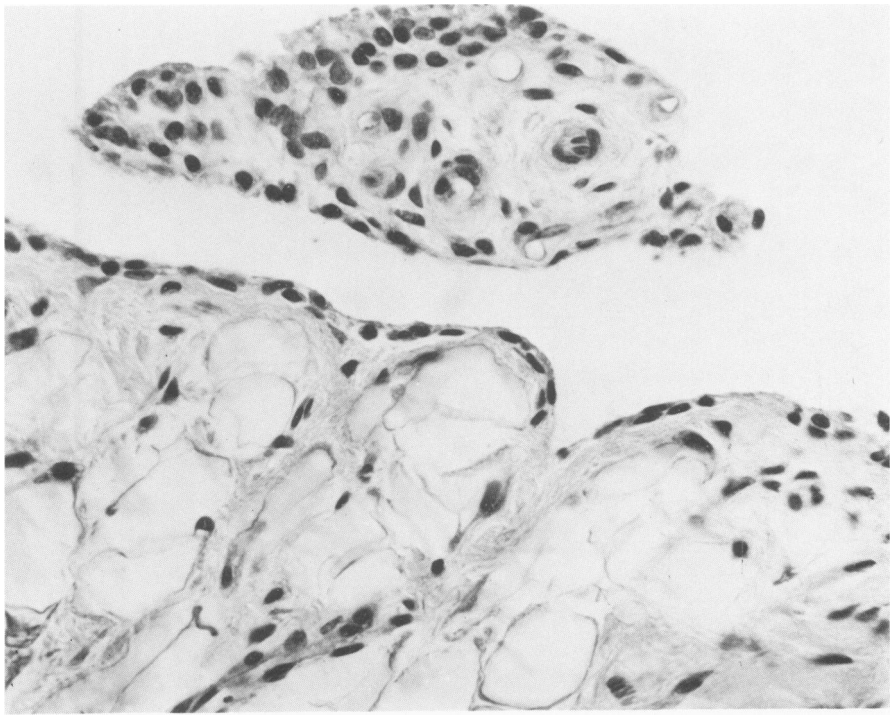
[*Illustrations follow*]



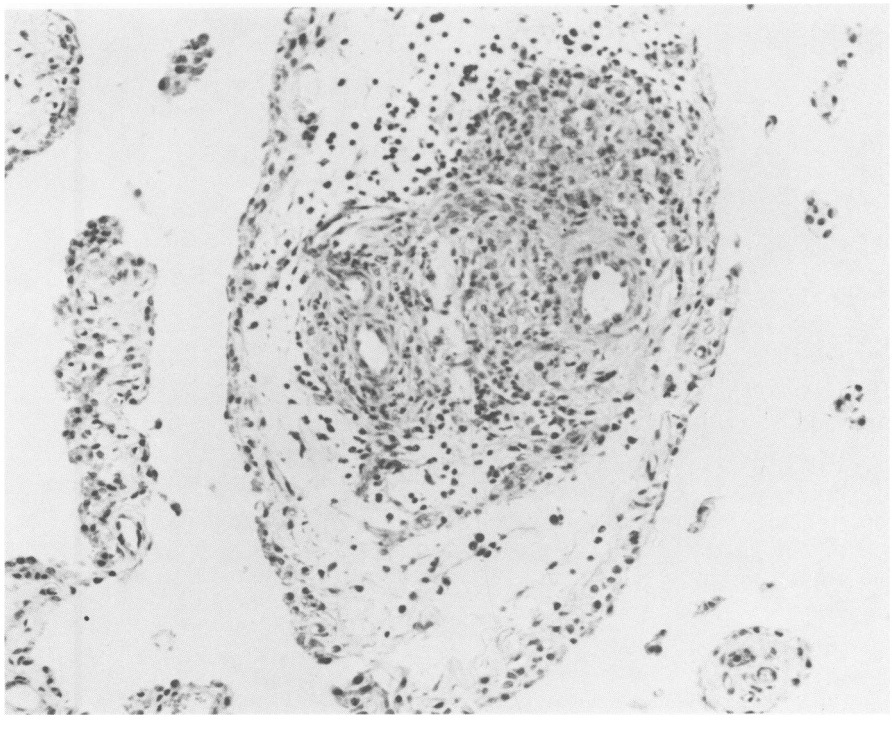
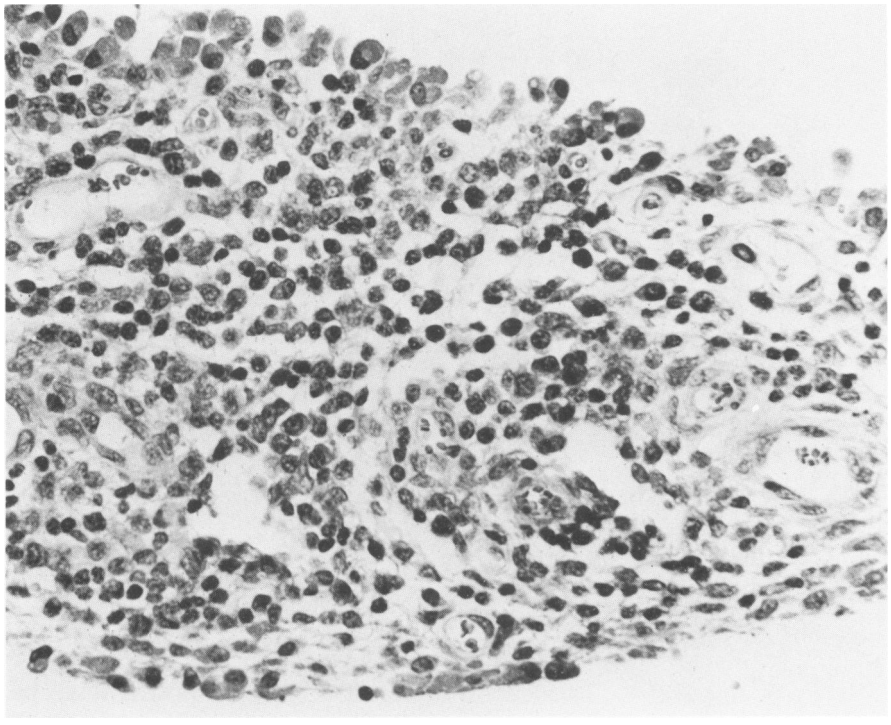


Figure 1—Synovial membrane from a normal uninoculated cesarean-derived kid. Note the paucity of inflammatory cells and low elongated profile of synovial cells. (H&E, ×400) **Figure 2**—Synovial villus from an inoculated joint at 6 DPI with edema, mild synovial cell hypertrophy, and perivascular mononuclear cell infiltrates. (H&E, ×160)



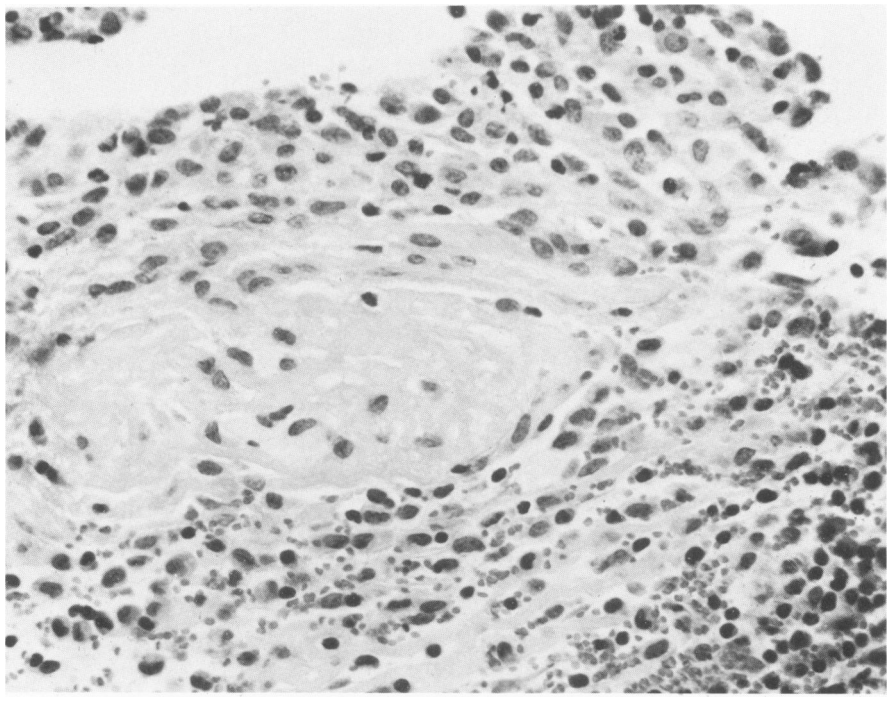
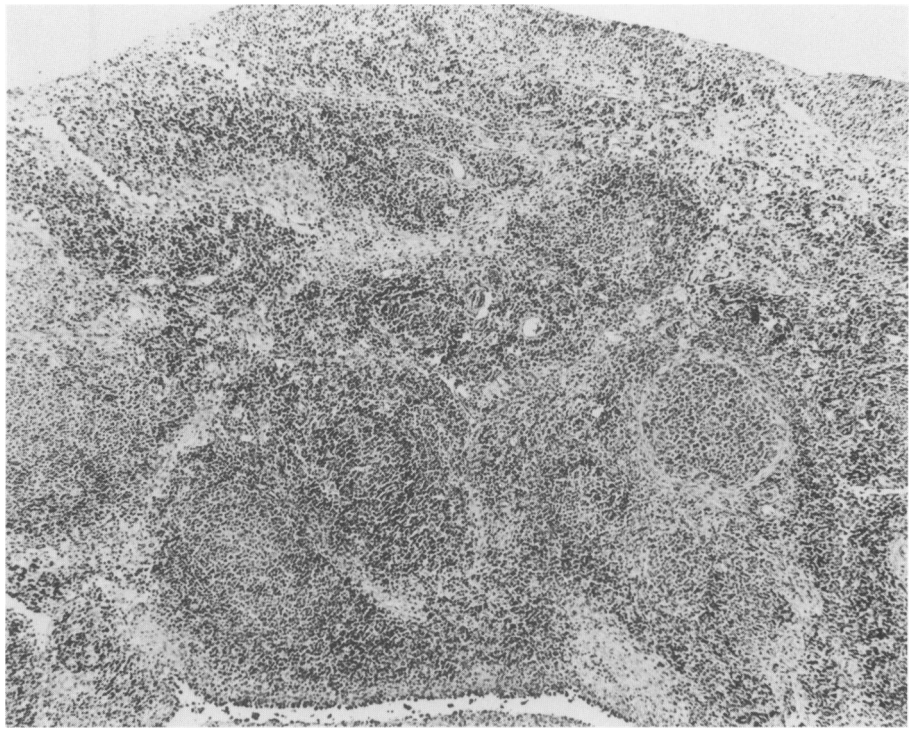


Figure 3—Synovial villus from an inoculated joint at 18 DPI, showing heavy infiltrates of lymphocytes, plasma cells, and macrophages, particularly around vessels, and synovial cell hypertrophy, hyperplasia, and separation from the connective tissue stroma of the villus. (H&E, ×400) **Figure 4**—Synovial membrane from an inoculated joint at 34 DPI with slight hemorrhage, fibrin deposition, and marked inflammatory cell infiltrates. (H&E, ×400)



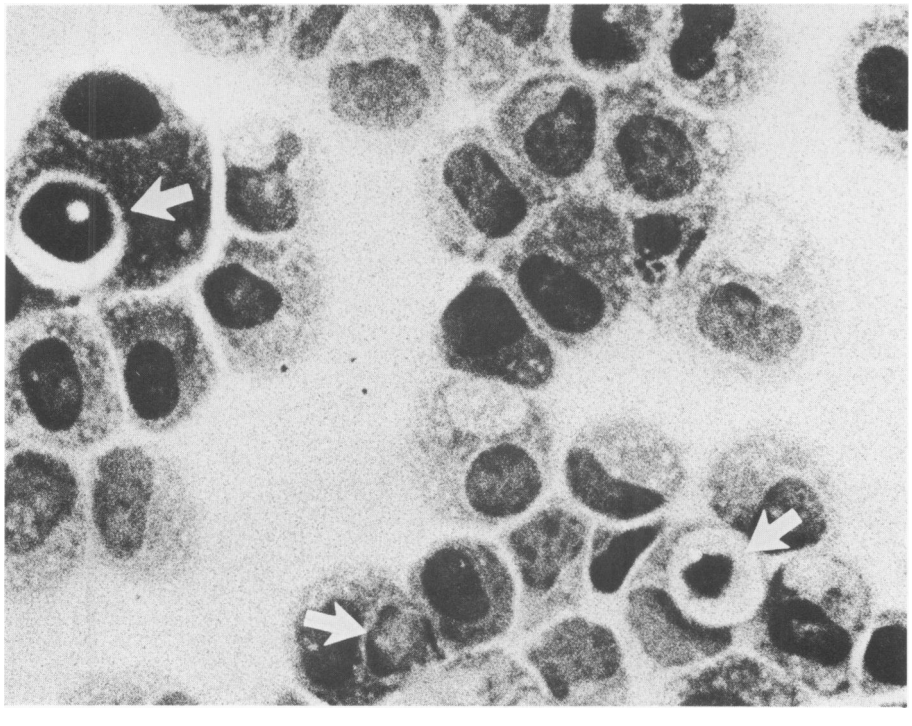
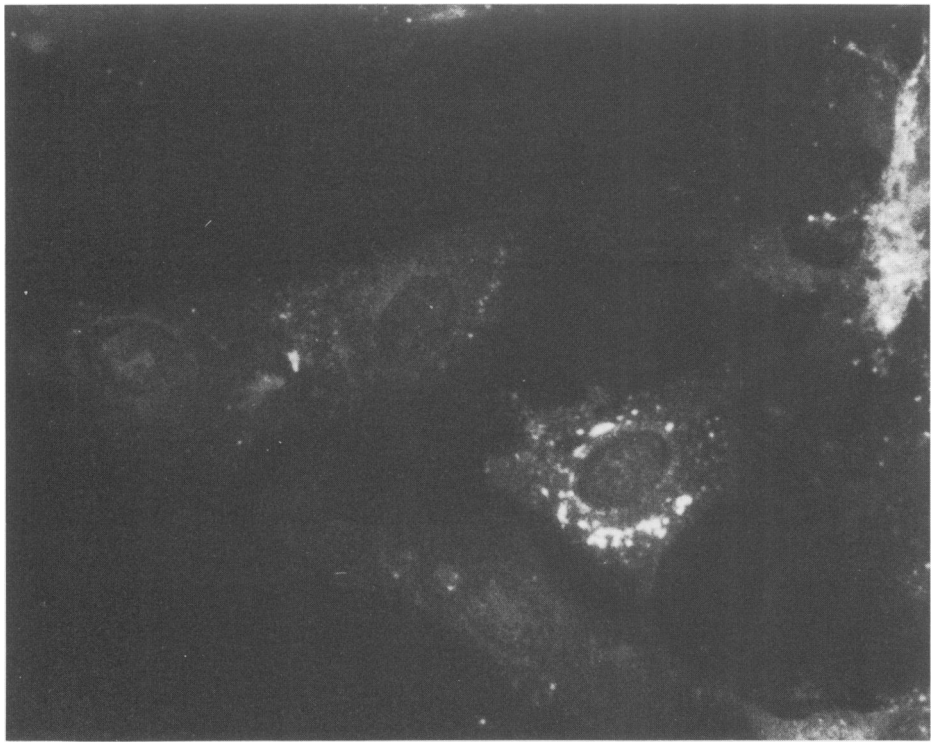


Figure 5—Synovial membrane from an inoculated joint at 45 DPI, showing heavy infiltration of inflammatory cells with formation of lymphoid follicles and germinal centers and significant thickening of the membrane. (H&E, ×32) **Figure 6**—Synovial fluid cells from an inoculated joint at 9 DPI made up primarily of large macrophage-like cells with foamy cytoplasm. *Arrows* show several that have engulfed other cells (phase-contrast). (H&E, ×800)



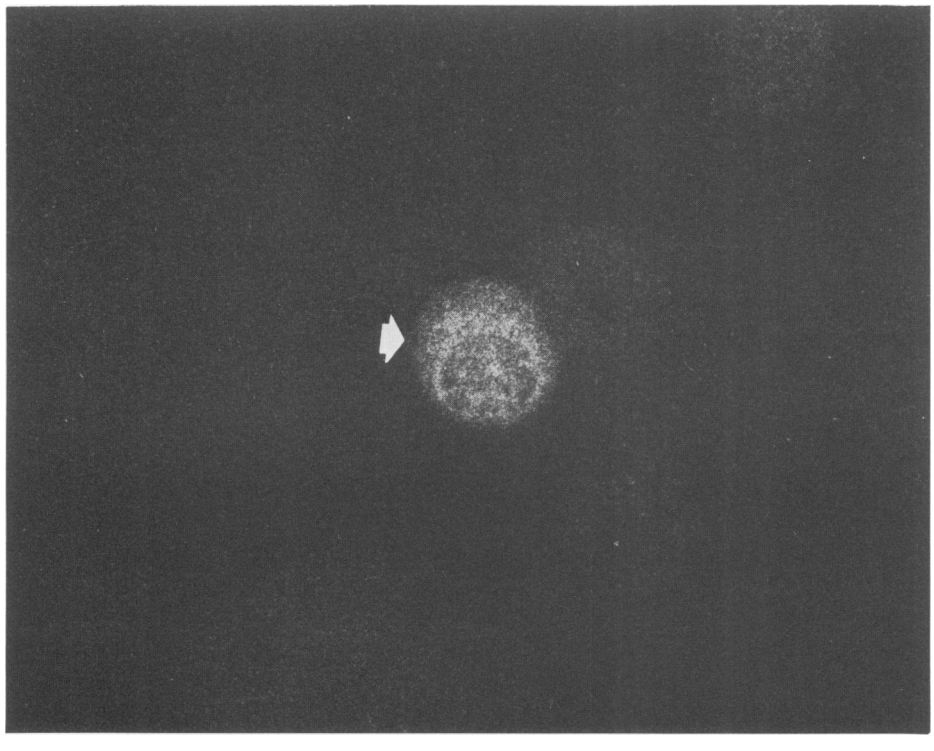


Figure 7—Fetal synovial membrane cells in culture, infected with CAEV and stained with anti-CAEV conjugate. Fluorescent viral antigen is particulate and cytoplasmic. Several negative cells are present as well. (×800) **Figure 8**—Synovial fluid cells from an inoculated joint at 2 DPI with faint finely particulate fluorescent viral antigen in one cell (*arrow*). Negative cells are barely visible. (×800)

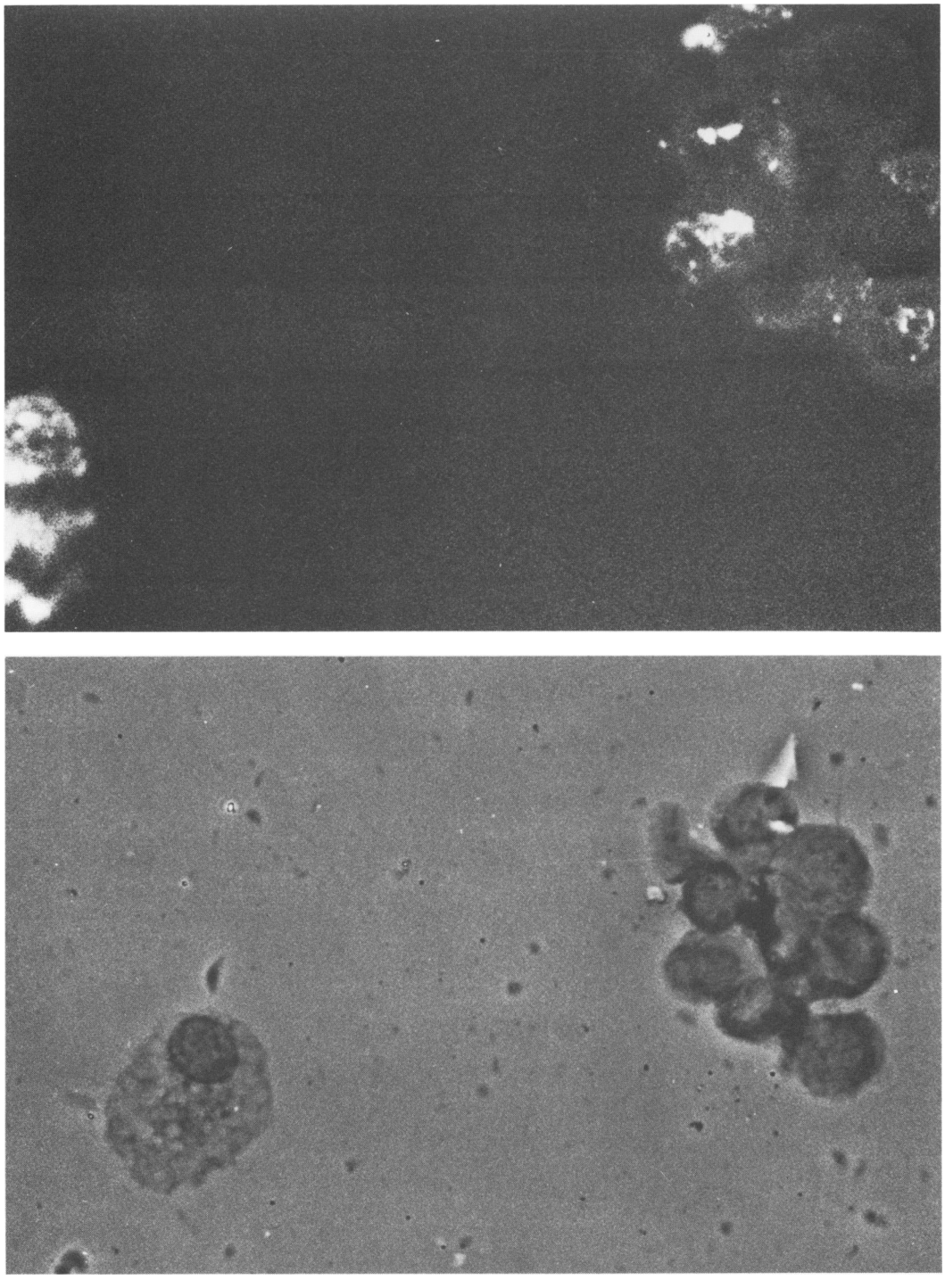
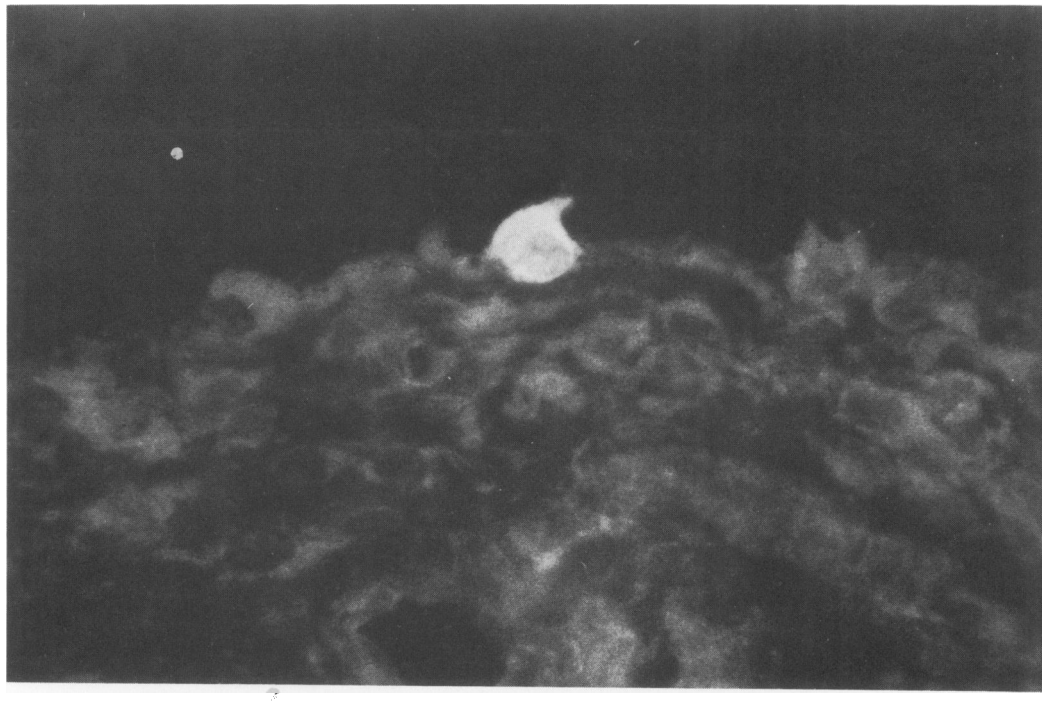


Figure 9—Synovial fluid cells from an inoculated joint at 6 DPI. Very bright nuclear and cytoplasmic fluorescent antigen. (×800) (With a photographic reduction of 11%) **Figure 10**—Cells from Figure 9 restained with H&E and photographed using phase-contrast microscopy. The morphology of the cells in the clump is difficult to interpret from the photograph, but the morphology of the large single cell is similar to that of the cells in Figure 6. (×650) (With a photographic reduction of 11%)



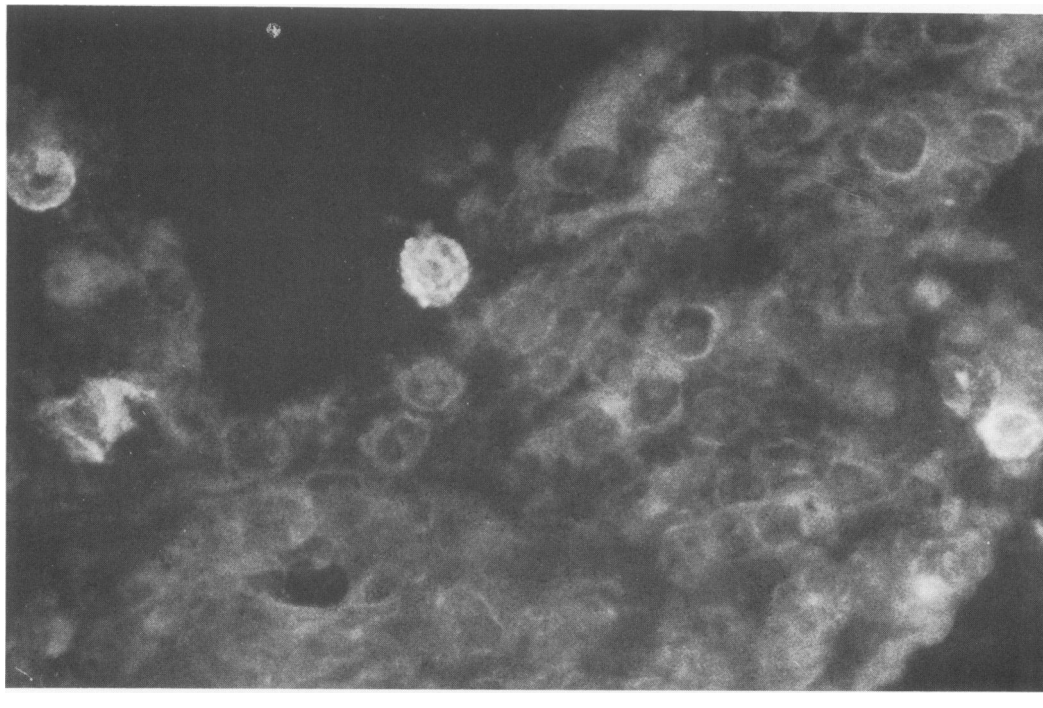


Figure 11—Synovial membrane from an inoculated joint at 9 DPI with a single cell containing fluorescent antigen at the synovial surface. (×800) (With a photographic reduction of 11%) **Figure 12**—Synovial villus from an inoculated joint at 9 DPI showing several cells at the edge of the membrane that contain fluorescent antigen. (×800) (With a photographic reduction of 11%)